THE

JOURNAL OF EXPERIMENTAL MEDICINE

THE JOURNAL OF EXPERIMENTAL MEDICINE

EDITED BY

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AND

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PUBLISHED FOR

THE ROCKEFELLER INSTITUTE FOR MEDICAL RESEARCH
NEW YORK

VOLUME FIFTEENTH

WITH EIGHTY PLATES AND FIFTY-THREE FIGURES IN THE TEXT

THE NEW ERA PRINTING COMPANY

1912

PRESS OF
THE NEW ERA PRINTING COMPANY
LANCASTER, PA.

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THE EFFECTS OF SUBDURAL INJECTIONS OF LEUCOCYTES ON THE DEVELOPMENT AND COURSE OF EXPERIMENTAL TUBERCULOUS MENINGITIS.*

By WILFRED H. MANWARING, M.D.

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The therapeutic control of tuberculosis has still to be accomplished. Of the specific therapeutic agents thus far proposed, tuberculin alone seems to exercise a favorable influence over certain forms of the disease. The results of sanatorium and climatic treatment, however, have amply demonstrated that there are in the body natural curative powers against this disease. While the mechanism of the natural cure has not as yet been fully worked out, it would nevertheless appear that phagocytic cells play an important part in it. It therefore becomes of interest to determine the extent of the action of the body phagocytes under controllable experimental conditions. The leucocytes are the phagocytic cells most readily obtained for experimental purposes.

Kling[1] has shown that leucocytes, injected simultaneously with tubercle bacilli, have the power of decreasing the pathogenic properties of these microörganisms. To Opie,[2] however, belongs the credit of a painstaking and systematic study of the effects of injected leucocytes upon a tuberculous lesion already developed. Opie inoculated the pleural cavity of dogs with tubercle bacilli, and found that leucocytes, brought into contact with the tuberculous tissue thus produced, made very striking alterations in it. The flat plaques of tuberculous tissue upon the pleura of the control ani-

* Received for publication, November 1, 1911.

[1] Kling, Untersuchungen über die bakterientötenden Eigenschaften der weissen Blutkörperchen, *Ztschr. f. Immunitätsforsch.*, 1910, vii, 1.

[2] Opie, The Effect of Injected Leucocytes upon the Development of a Tuberculous Lesion, *Jour. Exper. Med.*, 1908, x, 419.

mals, were almost absent in the treated dogs. The pleura was sometimes returned even to the delicate translucent appearance observed in the normal animal. The masses of tuberculous tissue in the mediastinum and in the sub-pericardial membranes were also smaller after the injections, and there were fewer metastatic lesions in the extrathoracic organs.

The work embodied in this paper is part of the study of the experimental therapeutics of meningeal infections,[3] which is being carried out at the Rockefeller Institute. The cerebrospinal membranes differ from the true serous membranes in that they are almost impervious to the blood proteids, and normally receive little or no protective substance from this source, and few, if any, leucocytes. The meningeal cavity is, therefore, always in an inferior condition of defense against infection. The method of lumbar puncture, however, provides a ready means of bringing healing substances into direct contact with pathological processes existing within these membranes, and has made possible a study of the local therapeutics of diseases of this surface. The experiments were made on dogs and consisted, first, in producing a tuberculous infection of the meninges, and, second, in attempting to control the lesion by the local injection of dog leucocytes.

METHOD.

Inoculation of the subdural cavity in the dog is attended with some difficulty. The spinal cord of this animal extends throughout the entire spinal canal, making the introduction of a needle, as for lumbar puncture, impracticable. Injury of the cord follows such introduction, and the resulting paralyses and other secondary effects rule out this method of procedure. A trephine opening in the cranium was therefore substituted.[4]

In devising the method, it was borne in mind, not only that inoculation of tubercle bacilli was to be provided for by this means, but also that provision must be made for the subsequent injection of

[3] Flexner, The Biological Basis of Specific Therapy, *Boston Med. and Surg. Jour.*, 1911, clxv, 709.

[4] All the operative procedures were carried out under morphin-ether anesthesia.

leucocytes. This was achieved by dividing the operation into two stages. In the first stage, the trephine opening was made, the button of bone removed was replaced by a disk of paraffin, the wound closed by cat-gut sutures, and allowed to heal under an antiseptic dressing. As soon as healing was complete, the actual inoculation was made, by passing a long, slender, blunt hypodermic needle through the skin, muscle, and paraffin disk, into and through the frontal lobe of the brain to the base of the skull. Leakage into the subcutaneous tissue was prevented by pressure on the paraffin on withdrawing the needle.

Preliminary experiments with suspensions of Berlin blue showed that a fluid so injected distributes itself quite uniformly over the base of the skull, extending a quarter, even a third of the way down the spinal meninges.

Several strains of tubercle bacilli were thus inoculated:

(1) Bovine. Originally obtained from Dr. M. P. Ravenel. Extremely virulent.

(2) Human (H39). Obtained from Dr. E. L. Trudeau. Virulent.

(3) Human (H24). Obtained from Dr. Theobald Smith. Slightly less virulent.

(4) Human (H38). Stock culture, Rockefeller Institute. Avirulent for dogs. Subdural injections of this culture at no time caused symptoms of meningitis. The bacilli gradually decreased in number and, while still present in considerable numbers as late as the fortieth day after injection, had disappeared by the sixtieth day.

The inoculation material was prepared by filtering emulsions of these cultures through closely packed absorbent cotton, and diluting the resulting filtrates to the desired turbidity. Bacterial emulsions so filtered are practically free from clumps. As a turbidity standard, a $\frac{1}{20}$ per cent. lecithin suspension was used, prepared by diluting a 1 per cent. alcoholic solution of lecithin with nineteen volumes of physiological saline. The volume of fluid usually injected was two cubic centimeters of the bacterial suspension, followed by one cubic centimeter of physiological saline to wash out the needle, a separate syringe barrel being used for the salt solution.

EXPERIMENTAL TUBERCULOUS MENINGITIS.

The injection of virulent tubercle bacilli into the subdural space of dogs by the above technique, produces a disease characterized by a latent or incubation period of from five to thirty days, followed by a period of increasing paralysis and incoördination that terminates, almost invariably, in death. The clinical picture, during the paralytic stage, varies in different animals, depending on the groups of muscles most involved, and on whether the paralyses are of the flaccid or of the spastic type. In order to record these paralyses, the following symbols have been adopted:

 = Apparently normal; no paralysis, no incoördination.

 = Beginning paralysis, beginning incoördination. Such an animal may show a slight spastic condition of the posterior extremities, or a tendency to stumble on running. A slight arching of the back and a beginning tenseness of all the trunk muscles are characteristic of this period.

 = Well-marked paralyses and incoördinations. Such an animal may have all its extremities either in a flaccid or a spastic condition. Its movements may be so incoördinate that the animal falls to its side; the neck and back may be held rigidly and the animal may cry out on being handled.

= Extreme paralysis, collapse. The animal in this stage is unable to stand. It usually lies on its side, refuses food, and rapidly emaciates. The back and neck are usually held rigidly.

= Death.

The following symbols have been adopted to represent symptoms unassociated with paralyses and incoördinations:

= Quieter than normally. The animal refuses to play, spends most of its time curled up in a corner of the cage, and may object slightly to being handled.

= Very quiet, weak, objects to being handled.

= Profound stupor, collapse. = Death.

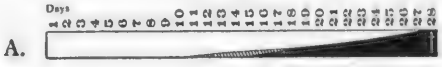
A typical course of the disease as represented by these symbols is shown in figure 1.

For the purpose of the present paper, the slight daily variations in the paralysis are of little moment. To obtain a less detailed

FIG. 1. TYPICAL COURSE OF TUBERCULOUS MENINGITIS. A fox-terrier, weighing 8 kilos, trephined 16 days previously. The wound healed by first intention. 0.1 c.c. of a suspension of bovine tubercle bacilli in 2 c.c. of physiological saline was injected, followed by 1 c.c. of salt solution to wash out the needle. The animal showed an incubation period of 11 days, mild constitutional symptoms beginning on the 12th day, definite paralysis on the 15th day, increasing to collapse on the 25th day; death on the 28th day. Autopsy showed a tuberculous process involving the entire meningeal cavity; abdominal and thoracic viscera negative.

picture, the above symbols have been combined,

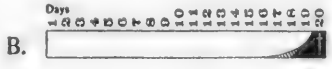
and the daily variation eliminated by changing the profile to a smooth curve.



This method of representation has been adopted in the subsequent parts of the paper.

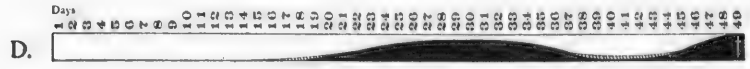
The above case, selected as the typical course of the disease, is characteristic of the majority of cases. About a quarter of the animals, however, do not conform to this type. For the sake of presenting these atypical cases, they are here divided into four groups. In these groups are included only uncomplicated cases of tuberculous meningitis, animals developing distemper and other intercurrent infections being carefully excluded from these records.

The first of these atypical cases is characterized by a slightly prolonged latent period, followed by sudden death, with or without slight preliminary paralytic symptoms. The animals are usually quieter than normally for a day or two before death. Death is usually preceded by convulsions and coma.

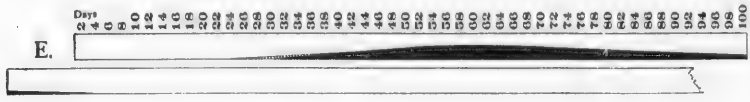


The second atypical group is characterized by an unusually prolonged paralytic stage. Animals of this type usually show the flaccid, rather than the spastic type of paralysis. The head and neck muscles are usually but little involved, thus interfering only slightly with the movements necessary for taking food.

The third group is characterized by a remission or actual disappearance of the paralytic symptoms, followed by their reappearance and death.



In the fourth group are included the spontaneous recoveries from the disease.



On account of the existence of these atypical cases, care must be exercised in drawing conclusions from clinical data, since parallel animals, inoculated with equal doses, may give quite different clinical pictures (figure 2).

To reduce these variations to a minimum, care was exercised in

FIG. 2. CLINICAL VARIATIONS. Two fox-terriers inoculated with equal doses (0.3 c.c.) of bovine tubercle bacilli. In the first animal (F), definite paralysis began on the 6th day, progressed to collapse on the 11th day; death on the 15th day. The second animal (G) showed no paralytic symptoms till the 20th day. Collapse on the 21st day; death on the 22d.

choosing animals for parallel inoculations. The animals selected were as nearly as possible of the same weight, the same breed, the same age, and in the same condition of nutrition. For most of the experiments, large, well nourished fox-terriers and bull-terriers were selected. Collies, spaniels, Irish terriers, and other long-haired dogs were avoided, as they were found irregular in their reactions and very susceptible to intercurrent infections. Young dogs were also avoided for the same reason. By exercising this care, it was found possible to produce approximately the same clinical picture in parallel inoculations (figure 3).

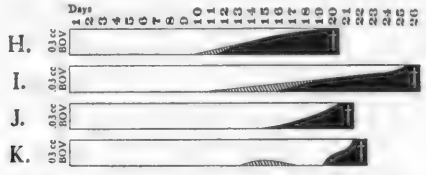


FIG. 3. PARALLEL INOCULATIONS. Four carefully selected adult fox-terriers, inoculated with equal doses of tubercle bacilli, showing comparatively slight variations in the clinical picture.

The clinical manifestations varied with the virulence of the culture injected (figure 4). With the very virulent bovine culture, the incubation period was short, the development of the paralytic symptoms rapid, and the outcome invariably fatal. With the less virulent human cultures, the incubation period was prolonged, the paralytic symptoms developed slowly, and an occasional animal recovered spontaneously.

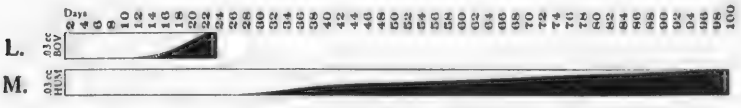


FIG. 4. RELATION TO VIRULENCE. Parallel inoculations with equal doses of bovine (L) and human (M) tubercle bacilli.

The clinical manifestations also varied with the dosage (figure 5). The latent period was perceptibly prolonged on decreasing

the dose, while with the smaller doses the paralytic symptoms developed slowly and there was a greater number of spontaneous recoveries.

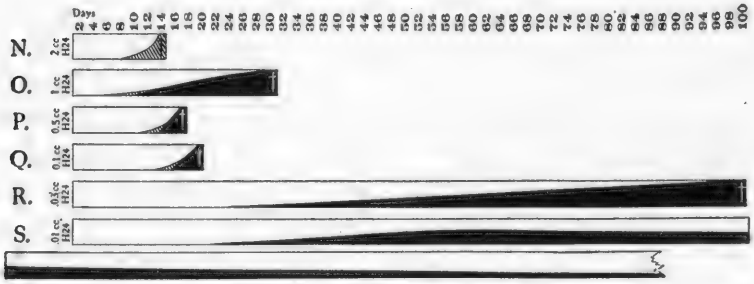


FIG. 5. RELATION TO DOSAGE. Parallel inoculations with decreasing doses of the same organism.

The local anatomical changes in the disease varied with the virulence and dosage of the organism injected. There was always more or less thickening of the meninges, with adhesions to adjacent structures. The subdural space usually showed an active tuberculous process, varying from an accumulation of fluid, fibrin, and semi-necrotic cells in the more acute cases, to well organized masses of diffuse tuberculous tissue in the more chronic cases. Smears made from the subdural space showed tubercle bacilli. There was usually a considerable accumulation of round cells about the cerebral and spinal capillaries, with occasional areas of necrosis.

The other organs of the body usually showed no characteristic change. Miliary tubercles were found in the livers of about a quarter of the dogs injected with the bovine culture, more rarely in the lungs and other organs. Such nodules, however, were never found in dogs injected with the human strains.

This does not necessarily mean that the bacilli of the human type did not occasionally escape from the meningeal cavity into the general circulation, but it indicates presumably only that the virulence of the human cultures was too small for the few that did escape to set up the disease in the extrameningeal tissues. This view is strengthened by experiments, in which small doses of the bovine

and human cultures were injected intravenously. With the bovine culture, a generalized miliary tuberculosis was always produced by this means. With the human cultures, such nodules were never formed.

Dogs recovering spontaneously from the paralytic symptoms showed, a few months later, simply a slight opacity of the meninges, with a few old adhesions. No tubercle bacilli were then found.

EFFECTS OF LEUCOCYTIC INJECTIONS.

The leucocytes used in treating the infected animals were obtained by the method perfected by Opie.[5] Five days before the proposed treatments, one cubic centimeter of turpentine was injected into the pleural cavity of a normal dog, and three days later a second injection of a similar amount was given. The resulting rapidly formed serous effusion was withdrawn and discarded on the fourth day, following which withdrawal a pleural exudate very rich in leucocytes was usually formed. This was used on the fifth day.

To prevent clumping, this exudate was drawn into about a third of its expected volume of 1.5 per cent. sodium citrate. The exudate was then filtered through gauze, freed from plasma by centrifugation, washed with salt solution, refiltered if necessary to remove lumps, and made up into a 50 per cent. suspension in physiological saline.

A microscopic examination of suspensions so prepared showed them to consist of mononuclear and polymorphonuclear leucocytes, mixed with a few red cells. Bacteria were never seen. Routine sterility tests, by transferring a sample to nutrient agar, gave uniformly no growth. About a third of the leucocytes stained immediately on the addition of very dilute methylene blue, from which it was concluded that these cells were probably necrotic, although still preserving their normal microscopic appearance.

In making the subdural leucocytic injections, the same technique was employed as in the initial subdural inoculations with tubercle bacilli; a long, slender, blunt hypodermic needle was passed through

[5] Opie, *loc. cit.*

the paraffin disk filling the permanent trephine opening, into and through the frontal lobe of the brain, and the leucocytes were forced into the basal meninges. The volume of suspension thus injected was usually about four cubic centimeters for fox-terriers, and six cubic centimeters for bull-terriers. Occasionally as much as ten cubic centimeters have been injected with apparently no ill effects.

The usual immediate effect of the injection was a temporary cessation of respiratory movements. As a routine, the animal was at once placed under artificial respiration, the tongue and epiglottis being retracted to insure a free respiratory passage. Spontaneous respiratory movements were usually resumed within two minutes, but were occasionally delayed for five or ten minutes, or even longer.

The later effects of the injections were less satisfactory. About a quarter of the animals died within a few hours. In a number of cases, death was attributable to edema of the lungs, or to unsuspected pneumonia from earlier etherizations; but many of the autopsies showed apparently no such accessory cause.

For several days after the injection, a number of the surviving animals were quiet, showed evidences of cranial tenderness, a few even showing disturbances in equilibrium, circulatory movements, and the like. These symptoms usually disappeared within two or three days, but occasionally persisted for a longer period. The majority of the animals, however, showed no symptoms the day after the injection.

The effect of the leucocytic injections varied with the dosage and virulence of the organism used. With large doses of tubercle bacilli the leucocytes were apparently without effect (figure 6).

FIG. 6. EFFECT OF LEUCOCYTIC INJECTIONS. Composite picture of four treated dogs (W) and four untreated controls (w). In making these composites, the average day on which symptoms of a certain severity first made their appearance was taken as the day of its appearance in the composite animal. The number of cubic centimeters of leucocytic suspension injected is indicated by the small exponents; thus, $L^6 = 6$ c.c.

With small doses of the very virulent bacilli of the bovine type (figure 7), the injections produced a uniform prolongation of the latent period of the disease, but had a less evident influence upon the subsequent course of the paralytic symptoms. Possibly the paralytic symptoms were somewhat less acute in the treated dogs than in the untreated controls, but the number of experiments was too small to establish this beyond question. All animals of this series died as a result of the meningeal infection.

With small doses of the less virulent human cultures, the injections had an even greater influence (figure 8). The control animals, inoculated with these doses, invariably developed paralytic and ataxic symptoms, usually after an incubation period of about four weeks. The resulting paralyses usually increased slowly in severity, and in half the cases terminated fatally in about three months. In the other half, the paralyses were of a more chronic type, persisting to the present date (seven months). Half of the animals receiving the leucocytic injections have shown no paralytic symptoms up to the present day (seven months), while the others have developed paralyses only after very prolonged incubation periods, from which one animal of the series has thus far died.

The local histological changes produced by the leucocytic injections can be determined only by killing animals at various stages after such injections. This has not been done. What can be stated, however, is that when fatal paralyses develop in the treated animals, the autopsy usually shows approximately the same histological changes as in the untreated controls. In some of the treated dogs, however, the tuberculous lesion in the upper parts of the subdural space has apparently been arrested, or caused to undergo resolution by the leucocytic injections, while persisting or advancing to a chronic condition about the lower third of the spinal cord, to which situation the leucocytic suspension presumably did not penetrate.

The study of the therapeutic control of tuberculous meningitis is made difficult in the dog by the necessity of making the injection and treatment through a trephine opening. Danger exists of producing fatal pressure effects, and the frequent etherizations expose the animal to intercurrent respiratory infections. Moreover, the

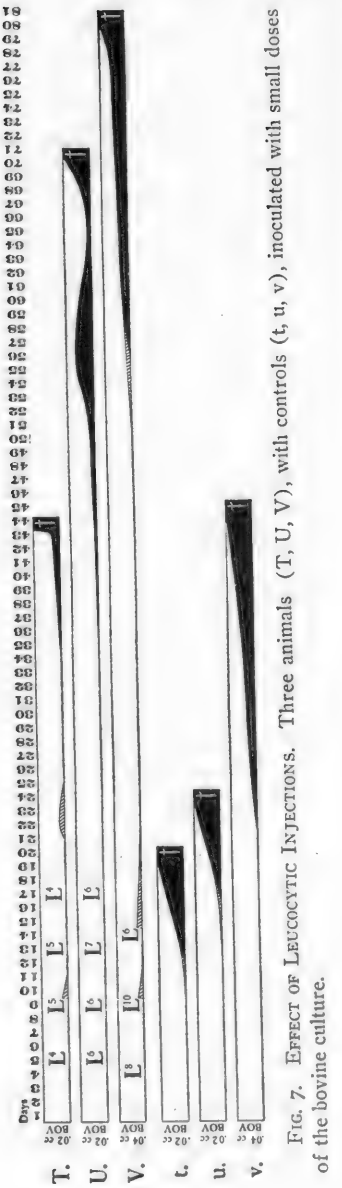


FIG. 7. EFFECT OF LEUCOCYTIC INJECTIONS. Three animals (T, U, V), with controls (t, u, v), inoculated with small doses of the bovine culture.

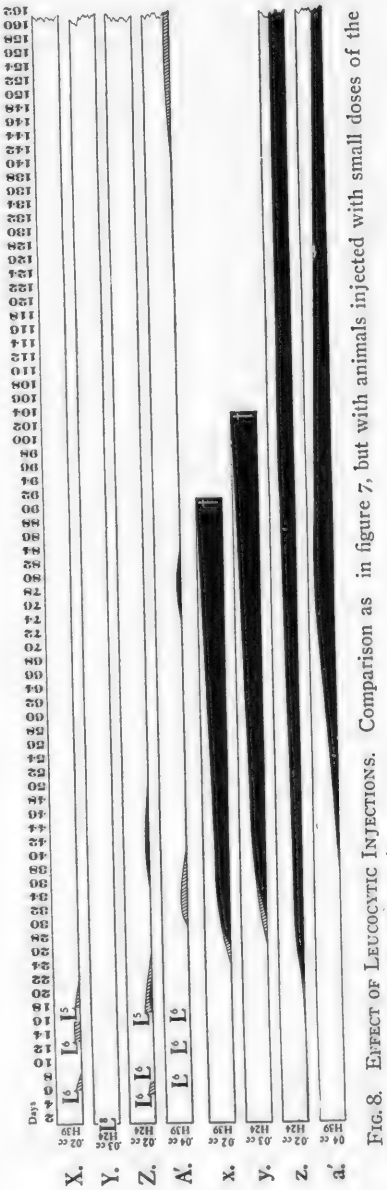


FIG. 8. EFFECT OF LEUCOCYTIC INJECTIONS. Comparison as in figure 7, but with animals injected with small doses of the less virulent bacilli of the human type.

medicament introduced into the cranial cavity penetrates with difficulty to the caudal levels of the spinal cord, leaving that part of the cord practically untreated. It is intended therefore to pursue this investigation upon animals in which the inoculations and injections can be made with less traumatic injury and under conditions more nearly approaching those occurring in spontaneous tuberculous meningitis in human beings.

The later history of the animals now under observation will be reported in a subsequent paper.

SUMMARY.

Subdural inoculation of tubercle bacilli of established virulence for guinea pigs and rabbits, produces in the dog a tuberculous meningitis, followed by paralysis and death. When suspensions of canine leucocytes are injected subdurally, following such inoculations there occurs uniformly a delay in the development of the paralysis and a prolongation of the life of the treated animal. In dogs inoculated with small doses of tubercle bacilli of low virulence, the development of paralysis has been prevented by this means for periods of seven months (up to the present date), while the untreated animals injected with the same cultures have all developed paralytic symptoms within a period of about four weeks.

STUDIES WITH THE ELECTROCARDIOGRAPH ON THE ACTION OF THE VAGUS NERVE ON THE HUMAN HEART.

II. The Effects of Vagus Stimulation on the Hearts of Children with Chronic Valvular Disease.*

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Plates 1–5.

During the study that forms the subject of the present communication, the methods and conditions were constantly similar to those described in our first paper (1). The electrocardiograms were always obtained by electrodes attached to the right forearm and left leg—the so-called second lead. The string of the galvanometer was adjusted so that its shadow moved one centimeter on the scale attached to the camera when a current of one millivolt passed through it.

The hearts of children suffering with so-called chronic endocarditis were found to be very susceptible to vagus stimulation by pressure. The study was carried out on a group of four children, in all of whom acute articular rheumatism can probably be held accountable for the heart lesions.

Case I. A girl of eight years, who first gave evidence of heart disease when six years old at the time of an attack of purpura. The diagnosis at the time of admission was mitral insufficiency, probably also aortic insufficiency and chronic pericarditis. The pulse rate ranged from 110 to 150 per minute while quiet in bed, and the blood pressure was estimated by the auscultatory method at systolic 105, diastolic 25 millimeters of mercury.

Case II. A boy of fourteen years, whose cardiac symptoms developed at the age of nine, two years after acute articular rheumatism. He suffered from mitral and aortic insufficiency. The pulse rate was from 100 to 126 per minute, and the blood pressure was systolic 110, diastolic 54 millimeters of mercury on admission, and on the next day the systolic pressure was 130.

*Received for publication, October 11, 1911.

Case III. A girl of seven years, who first showed cardiac symptoms during her fifth year after tonsillitis and diphtheria. Later she had acute articular rheumatism and presented the signs of mitral insufficiency. Her heart rate was 110 per minute on admission.

Case IV. A girl of fifteen years, who, besides having had rheumatism, gave a positive Wassermann reaction and whose parents gave a definite history of syphilis. Her cardiac symptoms appeared during her ninth year and the diagnosis of mitral insufficiency and congenital syphilis was made. Her pulse rate was 75 to 85 while quiet in bed, and her blood pressure was systolic 98, diastolic 60 millimeters of mercury.

In each case, the heart was beating with sufficient force to give a very well defined apex impulse, and in the first three cases, in which the rate was abnormally rapid, the heart action may be described as thumping. All the cases showed a marked enlargement of cardiac dullness on percussion. It is, of course, impossible to say what myocardial changes were present, but it is reasonable to suppose that the heart muscle, especially in the case where both rheumatism and congenital syphilis had been present, was at least physiologically abnormal. All the curves to be presented were obtained when the patients were free from the effects of digitalis.

The curves will first be described and the various features of the entire group will then be discussed.

The electrocardiograms have been analyzed in order to determine how the vagus stimulation has affected the various cardiac properties—rhythmicity, conductivity, contractility, and excitability—and the results of the analyses have been tabulated as previously. The measurements are given in seconds, calculated from the markings of the Jacquet chronograph, except those of the heights of waves which are in millimeters. The duration of each ventricular cycle is determined by measuring the time elapsing between the first evidence of ventricular stimulation in the electrocardiograms. This time is designated *Q–Q* or *R–R* in the tables, depending on whether the initial ventricular stimulation produced a negative *Q* wave or a positive *R* wave. The conduction time is measured by computing the time elapsing from the onset of auricular stimulation, the foot point of the *P* wave, to the onset of ventricular stimulation. This time is therefore represented by *P–Q* or *P–R* in the tables. In the same way, the length of auricular cycles is designated as *P–P*. Ventricular systole is measured from the onset of the *Q* or *R* wave to the end of the *T* wave, while from this last point to the following *Q* or *R* point represents ventricular diastole.

Figures 1 to 6 are from case I. Figure 1 shows one cycle of the electrocardiogram obtained when the heart was beating at its usual rapid rate, 140 per minute, undisturbed by vagus stimulation. The other eight cycles in this curve are very similar, and the measurements average as follows:

$$Q\text{-}Q = 0.430, \quad P\text{-}Q = 0.152, \quad P = 3.6, \quad R = 18.2, \quad T = 3.2.$$

No comment is needed except to call attention to the rapidity of rate, which causes a partial fusion of the *T* and *P* waves, and the prominence of these waves.

Figure 2 shows the effect of pressure on the right vagus nerve, figure 3 on the left vagus. The measurements from these curves are given in the following tables.

FROM FIGURE 2.

Curve 27.1. Right Vagus Pressure.

Number of cycle.	*Q-Q.*	Systole.	Diastole.	*P-Q.*	*P.*	*R.*	*T.*
1	——	——	——	0.152	2.5	15.7	2.2
2	0.458	——	——	0.157	3.0	16.5	2.2
*3	0.422	——	——	0.162	2.7	14.5	2.0
4	0.400	0.282	0.218	0.162	2.7	15.5	2.0
5	0.972	0.282	0.690	0.151	2.2	15.0	2.5
6	0.997	0.323	0.674	0.161	1.5	15.0	2.5
7	0.782	0.298	0.484	0.150	1.0	15.3	2.5
8	0.653	0.304	0.349	0.164	1.2	16.0	2.5
9	0.590	0.297	0.293	0.150	2.0	15.5	3.0
10	0.575	0.297	0.278	0.165	2.0	—	3.0
11	0.537	0.290	0.247	0.157	2.2	16.0	3.0
12	0.532	0.292	0.240	0.161	2.5	15.5	2.7
13	0.500	0.294	0.206	0.158	2.5	15.5	2.5
14	0.495	0.297	0.198	0.149	2.5	16.0	2.3

* Vagus pressure was made at the point of the first asterisk and was released at the point of the second asterisk in all curves where the release is indicated.

FROM FIGURE 3.

Curve 27.2. Left Vagus Pressure.

Number of cycle.	*Q-Q.*	Systole.	Diastole.	*P-Q.*	*P.*	*R.*	*T.*
1	——	——	——	0.149	3.0	18.5	3.0
2	0.462	——	——	0.153	3.2	18.0	—
*3	0.446	——	——	0.160	3.5	17.5	3.0
4	0.454	——	——	0.160	3.5	17.2	3.0
5	0.478	——	——	0.168	3.3	18.5	3.0
6	0.497	——	——	0.186	3.5	18.2	3.0
7	0.546	0.301	0.245	0.249	3.5	19.0	3.0
8	0.629	0.288	0.341	0.227	3.3	18.5	3.0
9	0.710	0.298	0.412	0.249	3.5	21.5	2.5
10	0.654	0.309	0.345	0.264	3.5	21.5	3.0
11	0.626	0.314	0.312	0.228	3.5	19.5	2.5
12	0.643	0.299	0.344	0.246	3.0	—	3.0
*13	0.600	0.300	0.300	0.217	3.5	20.0	3.0
14	0.535	0.310	0.225	0.183	3.5	18.5	3.0(?)
15	0.541	0.310	0.231	0.169	3.5	19.5	3.5
16	0.526	0.304	0.222	0.165	3.0	20.0	3.5
17	0.503	0.309	0.194	0.162	3.0	20.5	3.0
18	0.495	0.290	0.205	0.150	3.0	19.0	3.0
19	0.475	0.299	0.176	0.145	3.5	19.0	3.0
20	0.490	——	——	——	3.5	—	—

On another occasion, pressure was made first on the right vagus and then on the left with practically similar results, as seen by the following measurements of the curves which are not reproduced.

CURVE NOT REPRODUCED.

Curve 24.2. Right Vagus Pressure.

Number of cycle.	*Q–Q.*	*P–Q.*	*P.*	*R.*	*T.*
1	——	——	——	17.8	3.2
2	0.432	0.142	2.5	16.5	3.5
3	0.433	0.148	2.5	16.5	2.8
4	0.433	0.147	2.5	——	3.8
*5	0.439	0.157	3.0	17.5	3.2
6	0.479	0.155	3.0	17.3	3.4
7	1.768	0.217	1.7	19.0	3.5
8	0.657	0.211	1.8	18.5	4.5
9	0.653	0.175	1.8	18.0	4.0
10	0.711	0.192	2.0	19.0	4.5
11	0.719	0.164	2.0	18.8	4.3
12	0.635	0.167	1.8	18.2	5.0
13	0.592	0.133	2.0	18.2	5.0
*14	0.597	0.158	2.0	18.5	4.5
15	0.530	0.135	2.5	18.5	5.0
16	0.511	0.155	2.5	18.2	4.5
17	0.492	0.154	2.5	18.6	4.5
18	0.488	0.158	3.0	18.0	4.2
19	0.467	0.158	2.7	17.6	—

CURVE NOT REPRODUCED.

Curve 24.3. Left Vagus Pressure.

Number of cycle.	*Q–Q.*	*P–Q.*	*P.*	*R.*	*T.*
1	——	0.152	3.0	18.0	3.5
2	0.433	0.148	2.7	18.0	4.0
3	0.438	0.152	3.0	18.5	3.5
*4	0.436	0.154	3.0	17.0	3.5
5	0.431	0.151	3.0	18.5	3.5
6	0.438	0.153	3.0	18.5	4.0
7	0.453	0.167	3.0	18.5	3.5
8	0.524	0.223	3.5	19.0	3.0
9	0.522	0.219	3.0	19.0	3.0
10	0.542	0.208	3.0	19.0	3.5
11	0.472	0.190	3.5	19.0	4.0
12	0.462	0.177	3.0	19.0	4.0
13	0.464	0.184	3.5	18.0	4.0
14	0.463	0.169	3.0	18.0	4.0
15	0.482	0.166	3.0	18.5	——
*16	0.459	0.160	4.0	18.5	4.0
17	0.474	0.173	3.5	18.0	4.0
18	0.464	0.169	3.0	18.0	4.0
19	0.462	0.163	3.5	18.0	4.0

It is seen from the measurements in both sets of curves that there has been a marked difference between the effect of stimulation of the right and left vagus. Right vagus pressure had a striking effect on the heart rate, apparently through its action on the sinus node, the normal "pace-maker" of the heart-beat. The slowing came on less than a second after pressure was made and caused the maximal lengthening of the cardiac cycle in the first or second slow

beat. Left vagus pressure caused but slight slowing, and the longest cycle in each curve was the seventh after the pressure was exerted, each occurring about three seconds after the initial pressure. The time of conduction of the heart-beat from auricles to ventricles, as indicated by the *P–Q* time, is, however, more influenced, both relatively to the heart rate, and absolutely, by stimulation of the left vagus than the right. In the curve 27.1 (figure 2) right vagus pressure produced no delay in conduction, although the slowing of the cardiac rhythm was marked. Pressure on the left vagus nerve caused marked delay in conduction on both occasions, in curve 27.2 (figure 3) with moderate, and in the unpublished curve, 24.3, with almost no change in rate. The changes in conduction occurred synchronously in the curves with the changes in rate. Right vagus stimulation caused a striking diminution in the size of the *P* wave, which was apparently unaffected by left vagus pressure. The *R* wave was usually increased, slightly more by left-sided pressure, and the *T* wave more by right-sided, but not until several beats after pressure was made.

On two occasions in case I, right vagus pressure produced a dissociation of auricles and ventricles, seen in figure 4. The measurements from this curve are as follows:

FROM FIGURE 4.

Curve 19.2. Right Vagus Pressure.

Number of cycle.	*Q–Q.*	*P–Q.*	*P–P.*	*P.*	*R.*	*T.*
1	0.421	——	——	——	——	3.5
2	0.424	0.149	0.424	4.0	19.5	3.5
3	0.434	0.149	0.435	4.0	18.0	3.5
4	0.420	0.148	0.419	4.0	18.0	3.3
5	0.420	0.149	0.420	4.0	19.0	3.0
6	0.433	0.149	0.431	3.8	19.5	3.3
*7	0.670	0.151	0.658	3.8	18.8	3.7
8	0.687	0.170	0.661	2.5	20.8	3.5
9	0.991	0.196	1.011	3.0	21.2	3.8
10	°0.853	0.176	0.991	1.8	20.0	4.4
11	°0.788	——	1.000	——	20.0	5.0
12	°0.794	——	0.700	——	22.8	4.0
13	°0.778	——	0.706	——	21.8	4.5
14	°0.780	——	0.674	——	24.0	5.0
15	0.635	0.137	0.596	——	21.3	5.2
16	0.556	0.133	0.640	3.3	21.5	5.0
17	0.517	0.141	0.509	3.3	20.0	5.0
18	0.489	——	0.493	3.0	20.0	4.3

° = independent ventricular cycles. Independent ventricular rate = 75.1.

It is seen that after definite slowing in rate, but without maximal delay (as compared to figure 3), the *R* and *P* waves occur in abnormal relations in cycles 11, 12, 13, 14, and 15. The relations of the waves then resume that seen before vagus pressure was exerted, except for a shortening in the conduction time. It seems evident that this temporary disturbance in the electrocardiogram results from a complete dissociation of auricles and ventricles. The ventricles take up their own pace at a rate of 75.1 beats per minute, but the first independent ven-

tricular cycle is longer than those that follow. Practically the same ventricular complex is seen during as before and after the dissociation. The changes in the *R* waves in this part of the curve seem referable to the coincidences of the *P* and *R* waves. Otherwise the changes in the various waves are similar to those previously seen, except that there is a well defined increase in the *T* wave.

Figures 5 and 6 are reproduced to show the effect that right vagus pressure had on the cardiogram, taken by means of an open funnel held over the apex beat. The child had a thin chest wall, and the violently beating heart produced a very well defined apex beat. In figure 5, the cardiogram is recorded synchronously with the electrocardiogram. Soon after the initial pressure over the right vagus, which took place between the fifth and sixth cardiac cycles, the cardiogram becomes diminished in height until the thirteenth cycle is reached, when it is seen to have almost disappeared. The appearance of the entire length of this curve and of a number of others that were taken, make it certain that respiratory movements are in no way responsible for this flattening out of the cardiogram. Greatly weakened ventricular contractions seem to be the only possible cause. The marked diminution in intensity of the cardiac sounds and murmurs, which invariably occurred, confirm this explanation. The late appearance of the maximal weakening of the ventricles is worthy of note. It occurs over four seconds after the initial pressure and does not accompany the maximal slowing or depression of conductivity. The measurements from the curve show, however, neither a marked decrease in rate nor much delay in conduction.

FROM FIGURE 5.

Curve 19.5, with Apex Beat. Right Vagus Pressure.

Number of cycle.	*Q–Q.*	*P–Q.*	*P.*	*R.*	*T.*
1	0.436	0.149	—	——	—
2	0.430	0.154	3.5	18.0	3.5
3	0.437	0.152	3.5	18.0	3.5
4	0.432	0.150	3.0	18.0	3.5
5	0.442	0.154	3.5	18.0	—
*6	0.470	0.161	—	18.3	4.0
7	0.550	0.171	4.0	17.5	3.5
8	0.582	0.174	3.0	18.0	—
9	0.652	0.169	2.7	20.0	4.0
10	0.652	0.165	2.8	20.0	4.0
11	0.731	0.155	2.8	19.5	4.3
12	0.670	0.157	2.4	19.5	4.5
†13	0.637	0.160	2.5	21.0	—
14	0.635	0.156	2.0	20.5	5.5 (?)
15	0.560	0.155	3.0	19.0	4.5
16	0.556	0.168	2.5	20.0	5.0
17	[illegible]	0.155	3.0	19.5	4.5
18	—	——	2.7	19.0	4.0

† Greatest decrease in size of apex beat.

It is also interesting to find that the *R* wave of the greatest height occurs with the most weakened beat, while the greatest depression of the *P* wave is seen in the following beat. The definite increase in the *T* wave is seen in the latter part of the curve, as in the others, when the right vagus was pressed upon.

Figure 6 shows tracings from the apex beat and brachial artery, recorded in the usual way on the smoked paper drum of a kymograph. Right vagus pressure made while the curve was being taken, produced the same diminution in the cardiogram. The slowing of the heart responds to right vagus stimulation in what seems to be the usual way, while it is the second beat after the longest pause that forms the smallest cardiogram. The explanation for the fact that the weakest beat and the longest pause do not occur synchronously here may lie in the fact that the long rest afforded the heart had caused a stronger ventricular beat than occurred when the rest was not so long. This reasoning, however, can hardly be applied to explain the non-parallelism in rhythmicity and contractility which is seen in figure 5. Only a part of a long graphic record is reproduced, but when the entire curve was studied, respiratory effects could be excluded as the cause of the depression of the cardiogram. Cold and then hot applications to the neck of this patient, for about an hour and a quarter each, failed to alter the heart rate or the susceptibility of the heart to vagus pressure.

Figures 7, 8, 9, and 10 are from case II. In the record obtained without vagus pressure (figure 7), it is seen that the *P*, *R*, and *T* waves are very well marked, the *T* wave being abnormally large. Measurements of the entire curve of eight cycles, of which only part is reproduced, average as follows:

$$Q\text{–}Q = 0.553, \quad P\text{–}Q = 0.174, \quad P = 2.3, \quad R = 21.6, \quad T = 4.5.$$

The rate was 109 beats per minute. A slight depression of conductivity is indicated by the *P*–*Q* time of 0.174.

The difference in the effects of stimulation of the right and left vagi is seen by comparing figures 8 and 9.

FROM FIGURE 8.

Curve 41.2. Right Vagus Pressure.

Number of cycle.	*Q*–*Q*.	*P*–*P*.	*P*–*Q*.	*P*.	*R*.	*T*.
1	0.511	0.503	0.172	2.5	21.5	4.0
2	0.512	0.523	0.184	2.5	21.5	5.0
*3	0.572	0.584	0.173	2.5	22.0	4.5
4	0.834	0.811	0.161	2.5	22.0	4.5
5	0.777	0.799	0.184	1.0	22.5	4.0
6	0.789	0.792	0.162	1.0	22.0	4.2
7	0.823	0.815	0.159	1.0	22.0	4.5
8	0.800	0.805	0.167	——	22.5	4.5
*9	0.796	0.794	0.162	0.5	22.5	5.0
10	0.601	0.608	0.164	1.0 (?)	21.5	5.0
11	0.595	0.582	0.157	1.0	22.0	5.0
12	0.561	0.564	0.170	2.0	21.5	4.5
13	0.543	0.546	0.167	2.0	22.5	5.0
14	0.515	0.498	0.164	2.0	22.5	5.0
15	0.497	0.505	0.181	2.0	23.0	4.7
16	0.508	0.508	0.173	2.0	23.0	4.5
17	0.535	0.600	0.173	2.5	23.0	4.5
18	——	——	0.157	——	——	——

FROM FIGURE 9.

Curve 41.6. Left Vagus Pressure.

Number of cycle.	*Q–Q.*	*P–P.*	*P–Q.*	*P.*	*R.*	*T.*
1	0.524	0.520	0.171	2.5	23.0	4.5
2	0.524	0.522	0.175	2.5	22.3	4.5
*3	0.560	0.576	0.177	2.5	21.0	4.5
4	0.512	0.502	0.161	2.3	22.5	4.5
5	0.706	0.714	0.171	2.5	21.5	4.5
6	0.530	0.516	0.163	2.3	21.5	4.0
7	0.628	0.667	0.177	2.5	22.0	5.2
8	0.530	0.527	0.148	2.0	23.0	4.5
9	0.514	0.488	0.141	2.5	23.0	4.5
10	0.524	0.513	0.167	2.5	23.0	4.5
*11	0.612	0.631	0.178	2.5	23.0	4.5
12	0.554	0.551	0.159	2.0	22.0	4.5
13	0.531	0.539	0.162	2.0	22.5	5.0
14	0.534	0.517	0.154	2.0	22.5	4.5
15	0.530	0.524	0.171	2.0	21.5	5.0

With right vagus stimulation (figure 8), definite slowing occurred, but in a somewhat irregular manner. At the same time, no delay in conduction is apparent, but the striking diminution of the *P* wave, which nearly disappears, may have masked any lowering of conductivity. In those cycles showing almost an absence of the *P* waves, the *R* and *T* waves remain practically unchanged, so that it is the auricular portion of the electrocardiogram which is especially disturbed.

Left vagus stimulation (figure 9) produced only slight changes in the heart-beat in the one instance obtained. The only apparent influence on conduction is indicated by shortening of the *P–Q* time seen in the eighth and ninth beats, an effect exactly opposite to that seen in case I with left vagus pressure. No changes of note are seen in the *R* and *T* waves and only slight diminution occurs in several of the *P* waves.

The main interest in this case lies in the phenomenon of dissociation which was produced by right vagus pressure. It is seen in figure 10 that following vagus stimulation, a moderately prolonged cardiac cycle occurred, followed by a greater lengthening. At the end of this second lengthened cycle, 1.6 seconds after the initial pressure, there is a slight movement upward in the curve resembling the beginning of a *P* wave, which is interrupted by an entirely new type of ventricular complex consisting of a somewhat diminished *R* wave, a much exaggerated negative *S* wave, and a much enlarged *T* wave. This complex occurs in a series of three beats, each preceded closely by a small wave, evident from its position and shape as at least part of the *P* wave. The close proximity of the beginning of these *P* waves to the *R* waves makes it also evident that the auricles are not, during these three beats, the pace-maker for the ventricles. The abnormality of the ventricular complexes indicates that the stimulus of cardiac contraction does not pass through the ventricles in the usual way from the atrioventricular bundle, but that some abnormal point in the ventricles has taken up the pace-making function. The auricles and ventricles are beating

independently, therefore, during the sixth, seventh, and eighth cycles, each portion of the heart at its own rate under the conditions produced by right vagus stimulation. From the measurements from this curve, it is seen that the ventricular rate is 78.5 per minute. Measurements from the rather poor brachial tracing and the electrical curve indicate that the length of systole is about the same, whether the ventricular beat is of normal or abnormal type.

FROM FIGURE 10.

Curve 41.3. Right Vagus Pressure. Dissociation.

Number of cycle.	*Q–Q.*	*P–P.*	*P–Q.*	*P.*	*R.*	*T.*
1	0.502	0.502	0.165	2.5	22.0	4.5
2	0.490	0.480	0.165	2.5	21.0	4.5
3	0.494	0.485	0.175	2.5	21.0	4.5
*4	0.562	0.592	0.184	2.5	22.0	5.0
5	°0.753	0.881	0.154	1.5	——	5.0
6	°0.764	0.750	——	——	13.0	7.0
7	°0.776	0.714	——	1.0	13.0	7.0
8	0.668	0.629	—— (0.108)	1.0	14.0	7.0
*9	0.603	0.598	0.147	0.5	22.0	5.5
10	0.598	0.568	0.152	1.0	22.0	5.0
11	0.544	0.544	0.159	2.0	21.5	5.0
12	0.534	0.526	0.159	2.5	22.0	5.0
13	0.516	0.509	0.167	2.0	21.5	5.0
14	0.530	0.527	0.174	2.5	21.7	4.5
15	0.516	0.504	0.177	2.0	21.5	4.5
16	——	——	0.179	2.5	22.0	5.0

° = independent ventricular cycles. Independent ventricular rate = 78.5.

The brachial tracing gives no indication that these dissociated ventricular beats produce a weaker pulse than the others.

It should be noted that before the dissociation no appreciable delay in conduction is seen, while, when the auricles again resume their pace-making function, the conduction, as indicated by the *P–Q* time, is distinctly shortened, just as it is in figure 4. Here the flattening of the *P* waves, which is not seen in figure 4, may make this shortening only apparent, although it is quite reasonable to assume that the rest afforded the conducting system during the dissociation has rendered it capable of more rapid conduction than before.

The curves of especial interest from case III are those shown in figures 11 to 15. The undisturbed electrocardiogram (figure 11) is characterized by an especially prominent *T* wave. The heart rate calculated from the curve was 94.8 per minute, and a slight so-called sinus arhythmia is seen. The measurements average as follows:

$$R\text{–}R = 0.633,\quad P\text{–}R = 0.163,\quad P = 2.5,\quad R = 13.2,\quad T = 4.0.$$

Curves 66.2 and 66.4 (figures 12 and 13) demonstrate the difference in the effects of right and left vagus stimulation in this case.

FROM FIGURE 12.

Curve 66.2. Right Vagus Pressure.

Number of cycle.	*R–R.*	*P–P.*	*P–R.*
1	0.586	0.576	0.186
*2	0.642	0.663	0.188
3	°1.000	1.229	0.180
4	0.966	0.730	——
5	0.574	0.578	0.177
6	0.556	0.550	0.170
*7	0.597	0.608	0.176
8	0.576	0.568	0.165
9	0.597	0.592	0.173
10	0.598	0.603	0.178
11	0.592	0.592	0.173
12	0.593	0.586	0.173

° = independent ventricular cycle. Independent ventricular rate = 60 per minute.

FROM FIGURE 13.

Curve 66.4. Left Vagus Pressure.

Number of cycle.	*R–R.*	*P–P.*	*P–R.*
1	——	——	——
2	0.526	0.523	0.190
3	0.530	0.536	0.193
4	0.550	0.550	0.189
*5	0.541	0.527	0.194
6	0.544	0.544	0.208
7	0.544	0.570	0.208
8	0.549	0.534	0.182
9	0.550	0.555	0.197
10	0.550	0.558	0.192
*11	0.550	0.551	0.184
12	0.556	0.559	0.183
13	0.574	0.560	0.180
14	0.558	0.562	0.194

Again the difference in the action of the right and left vagus appears. Stimulation of the right nerve has slowed the heart rate and not affected conductivity; stimulation of the left nerve has produced exactly the reverse effect, although the delay in conduction is slight. No striking changes are seen in the sizes of the various waves in either curve.

Dissociation of auricles and ventricles occurs in the right vagus pressure curve. The *P* wave of the fourth cycle follows the *R* wave closely, falling between it and the *T* wave, thus indicating that auricular and ventricular systole occurred synchronously. Except for the distortion which this coincidence causes, and a slight increase in the height of the *R* wave, the ventricular complex retains its usual form, and it may be inferred, therefore, that the stimulus of ventricular contraction was propagated through the ventricles from the atrio-ventricular bundle in the normal course. The independent ventricular systole

occurs exactly one second after the preceding ventricular systole, showing an inherent ventricular rate of sixty per minute. The impairment of conductivity seen in the first part of the curve may be the result of a course of digitalis which had lasted nine days but had been discontinued five days before the curve was taken.

Another example of the difference in the effect of right and left vagus stimulation in this case is seen in curves 77.5 and 77.4 (figures 14 and 15).

FROM FIGURE 14.

Curve 77.5. Right Vagus Pressure.

Number of cycle.	*R–R.*	*P–P.*	*P–R.*	*R.*
1	0.526	0.557	—	9.0
2	0.529	0.517	0.163	9.0
3	0.501	0.507	0.175	9.3
*4	°1.081	1.389	0.169	9.0
5	1.060	1.175	—	10.0
6	0.475	1.144	—	10.0
7	°1.000	—	0.215	10.0
*8	0.988	0.894	—	9.5
9	0.697	0.684	0.142	9.0
10	0.626	0.624	0.155	9.0
11	0.562	0.562	0.157	9.0
12	0.547	0.530	0.174	9.0
13	0.539	0.537	0.176	9.0
14	0.526	0.525	0.175	9.0
15	0.537	0.544	0.168	9.0
16	0.549	0.557	0.160	9.0

° = independent ventricular cycles. Average length of independent ventricular beats = 1.047 seconds. Rate of independent ventricles = 57.3 per second.

FROM FIGURE 15.

Curve 77.4. Left Vagus Pressure.

Number of cycle.	*P–P.*	*P–R.*	*R.*
1	——	——	8.3
2	0.494	0.177	8.5
3	0.494	0.177	8.2
4	0.524	0.175	8.0
*5	0.528	0.180	8.0
6	0.542	0.187	8.2
7	0.558	0.185	8.0
8	0.545	0.172	8.3
9	0.554	0.180	9.3
10	0.580	0.197	8.7
11	0.584	0.184	9.0
12	0.548	0.163	9.0
*13	0.532	0.164	8.5
14	0.538	0.173	8.5
15	0.510	0.170	8.5
16	0.538	0.180	8.5
17	0.534	0.165	—

Although the electrocardiograms are somewhat unsatisfactory, the accompanying cardiograms from the apex beat make the curves of interest. The exceptionally pronounced apex beat gave very perfect records through the usual funnel receiver, so that it seems justifiable to consider the sudden decrease in size of the cardiograms as evidence of weakened ventricular contraction. The modifying influence of respiratory movements was excluded, and the diminution in intensity of heart sounds and murmurs lent further justification to our interpretation of the small cardiograms. Although no attempt is made to express numerically the difference between the cardiograms in the two curves, it is seen in the curves that left vagus pressure has caused apparently a greater decrease in the force of ventricular contraction than right vagus pressure. Consideration must be given to the fact, however, that left vagus stimulation has caused almost no effect on rate in distinction to the marked slowing that followed pressure on the right nerve. This difference in rate may be responsible for the difference in the cardiograms, for the long diastoles following right vagus pressure would allow time for recovery of the depressed contractility of the ventricles, which could not take place with the slightly reduced rate following left vagus pressure. Again it is seen, as pointed out in curve 19.5 (figure 5), that the *R* wave is increased in height in those beats where the greatest reduction in size of the cardiograms occurs, especially with left vagus pressure.

In the curve showing the striking dissociation (figure 14), the ventricles take up their own inherent rhythm in three beats and show a ventricular rate of 57.3 beats a minute, nearly the same as that seen in the curve (figure 12) taken two weeks previously. The ventricular rhythm is broken into by one beat coming through from the sixth auricular systole. The point on the curve when this auricular contraction occurs cannot be determined with certainty, but it seems to be coincident with the *T* wave of the preceding cycle and is followed after a long conduction time by the ventricular contraction. The length of conduction here probably results from the auricular contraction occurring toward the end of the preceding ventricular refractory phase. The ventricles, therefore, could not respond immediately to the stimulus from the auricles. That this lengthening is not the direct result of vagus stimulation seems probable, too, when it is observed that immediately after the dissociation, the conduction time is strikingly shortened and remains so for several beats. The conduction time is, however, lengthened in the left vagus pressure curve with almost no slowing of rate.

The curves from case IV (figures 16, 17, and 18) show a somewhat different type of reaction of the heart to vagus stimulation. Clinically the case differed from the others in that the rate was slower, the heart-beat less forcible, and the difference between systolic and diastolic blood pressure not so great. In curve 38.3 (figure 16), it is seen that the undisturbed electrocardiogram from this case differs distinctly from those of the preceding cases. The rate (75.7 per minute) is slower, and the *P* and *T* waves are distinctly less prominent. The measurements average:

$$R\text{–}R = 0.792, \quad P\text{–}R = 0.167, \quad P = 1.4, \quad R = 17.0, \quad T = 2.1.$$

Right vagus pressure (figure 17) produced moderate slowing of the heart

and slight lengthening of the conduction time, while the most striking feature of the curve is the great reduction and almost total disappearance of the *P* wave during the time of vagus stimulation. The *R* waves show a tendency to increase and the *T* waves to decrease in height during this time. After the removal of the pressure, the heart rate increases and the cardiac cycles become distinctly shorter than those seen before the pressure was made and than those in the curve unaffected by vagus stimulation (figure 16). At the same time, the *P* waves and the *T* waves are slightly higher than in the unaffected curve. This is seen especially in cycles 12 and 13, while in cycles 11 and 12 the *R* waves are slightly reduced.

FROM FIGURE 17.

Curve 38.4. Right Vagus Pressure.

Number of cycle.	*R–R.*	*P–R.*	*P.*	*R.*	*T.*
1	0.810	0.190	1.3	18.0	2.3
*2	1.264	0.178	1.0	18.0	2.0
3	1.200	0.184	?	18.3	1.0
4	1.257	0.192	?	19.0	2.0
5	1.306	0.203	?	18.0	2.0
6	1.069	0.193	?	19.0	2.0
*7	1.074	0.178	?	18.0	2.0
8	0.997	0.170	1.0	18.0	2.5
9	0.868	0.178	1.0	18.0	?
10	0.719	0.167	?	17.0	2.5
11	0.636	0.170	1.0	16.0	3.0
12	0.663	0.176	1.5	16.5	3.0
13	0.647	0.173	1.5	18.0	3.0
14	—	0.176	—	—	—

FROM FIGURE 18.

Curve 38.5. Second Lead. Left Vagus Pressure.

Number of cycle.	*R–R.*	*P–R.*	*P.*	*R.*	*T.*
1	0.740	0.155	2.0	16.0	2.8
2	0.766	0.169	1.0	16.0	2.0
*3	1.103	0.161	1.5	16.0	2.3
4	1.273	0.197(?)	?	17.0	2.0
5	1.162	?	?	16.0	1.5
6	1.018	0.183	1.0	16.5	1.5
*7	1.017	0.196	0.5	16.5	2.0
8	0.868	0.193	1.0	16.0	2.0
9	0.856	0.175	1.0	16.5	1.5
10	0.469	0.180	1.0	16.0	—
11	0.952	—	—	21.5	—
12	0.836	0.183	1.5	16.5	1.5

Left vagus pressure (figure 18) produced almost similar effects. The slowing is not quite so great, while the delay in conduction of the heart-beat from auricles to ventricles is about the same. The striking flattening of the *P* wave and the tendency for the *R* wave to be increased and the *T* wave to be decreased

in height are seen here also. After the removal of pressure, there is, however, no quickening of the heart rate and no increase in the *P* and *T* waves or decrease in the height of the *R* wave. Otherwise in this case in the curves presented and in several others, no definite difference could be made out in the effects from stimulation of the right and left vagus, although well marked effects were produced by pressure over each nerve.

DISCUSSION.

In the discussion of the curves that have been described, two features, the differences in the action of the right and left vagi and the significance of the dissociation of auricles and ventricles, demand special emphasis.

In two of these cases of so-called chronic endocarditis, a definite difference between the action of the two vagi has been constantly demonstrated. The control of rate predominated in the right vagus nerve, presumably through its inhibitory action on the normal pacemaker of the heart-beat, the sinus node. The control of conduction of the heart-beat from auricles to ventricles predominated in the left vagus nerve, presumably through its inhibitory action directly on the conducting system. It will be brought out later that the dissociations following right vagus stimulation depend primarily on the ability of this nerve to reduce auricular rate. The factor of heart rate must be reckoned with when the difference in the effect of the right and left vagi on conductivity is considered. The prolonged cardiac cycles resulting from right vagus stimulation afford an opportunity for recovery of the conductivity not given to the more rapidly beating heart when the left vagus is stimulated. An examination of the relation of the conduction times to the lengths of the preceding diastoles shows, however, that this difference in rate is at least only a contributing factor, and that the left nerve acts especially on conductivity.

There is sufficient evidence in the curves and in the auscultatory phenomena that accompanied the other vagus effects for the belief that in two of the cases vagus stimulation acted directly on the ventricles, causing a marked diminution in the force of their contractions. From the one case where a comparison of the effects of the two nerves on the contractility was made, no definite conclusions can be drawn as to their relative effectiveness, although

each nerve apparently lessened definitely the ventricular contractions. This effect of vagus stimulation was not studied in the other cases of this series. The curves furnish no evidence as to changes in the excitability or tonus of the heart.

The heart seems to respond not infrequently more quickly to stimulation of the right than of the left vagus. Often the maximal effects occur in the first or second beat after right vagus pressure becomes effectual, while they occur several beats later with left vagus pressure.

The influence which stimulation of the vagi has on the various waves of the electrocardiogram shows also a difference in the action of the two nerves. The most constant change in the form of the electrocardiogram is the reduction in the size of the *P* wave, which sometimes entirely disappears. The *R* wave is usually increased in height, and the *T* wave may be increased a number of beats after vagus stimulation has been made, or, as in one case, diminished synchronously with the diminution of the *P* wave. The *P* waves, which represent the auricular activity, were practically always more affected by right vagus stimulation than by left, while in the cases where the *R* waves, which represent the initial activity in the ventricles, were increased, this change was greater after left than after right vagus pressure. The greatest changes in the *P* and *R* waves usually occurred in the longest cardiac cycle or in the following cycle. It is noteworthy that in each of the three curves in which the cardiogram is also shown (figures 5, 14, and 15), the *R* waves reach their greatest heights in the heart-beats in which the weakest ventricular contractions are seen. This fact indicates that an increase in the *R* wave in the electrocardiogram cannot be taken to mean an increase in the force of contraction of the ventricles. The observation agrees with that of Hering (2), who found that when pulsus alternans was experimentally produced in animals, the weaker ventricular contractions gave a larger *R* wave than did the stronger contractions.

The difference in the action of the two vagi have not been constantly demonstrable by the somewhat uncertain method of vagus stimulation which we have employed. In case IV, pressure on each nerve produced striking but practically similar effects, and in an-

other case quite contrary results were obtained in the one comparison that was made of the difference in action of the two nerves. This patient was a man of twenty-nine years with chronic endocarditis. Left vagus pressure was followed by greater slowing of rate but by less delay in conduction than right vagus pressure. The effects of vagus pressure were comparatively slight in each instance. The significance of this finding is not clear, but it indicates that an investigation of many more patients is necessary to determine with what constancy differences in the action of the two vagi can be demonstrated in man by means of vagus pressure.

In our first communication, we described the effect of right vagus stimulation on hearts with auricular fibrillation, and showed that long ventricular pauses occurred without any appreciable change in the auricular fibrillation. This effect was attributed to a lowering of conductivity to such an extent that all the impulses which would ordinarily reach the ventricles from the fibrillating auricles were blocked. At first sight, it would seem that in these cases the right vagus was capable of very marked effect on the conductivity, but when it is remembered that a large majority of the rapidly formed impulses arising in the auricles are ordinarily blocked, it is reasonable to assume that complete blockage for several seconds may be produced by the slight further lowering of conductivity of which the right vagus is capable. No comparative studies of the two nerves were made in the fibrillating cases, so it is not possible to say whether stimulation of the left vagus could have been more effectual even than stimulation of the right. We wish to emphasize the fact, however, that in these cases with auricular fibrillation, marked effects of right vagus stimulation on the ventricular rate, probably due to changes in the conductivity of the heart, can apparently be demonstrated, while in them no opportunity is afforded for conclusions as to the influence of the nerve on the rhythmicity of the heart.

There are, therefore, facts which do not seem to agree entirely with the findings in the cases where differences in the two vagi are clearly demonstrated. However, it seems probable to us that usually in man, in healthy as well as in diseased hearts, the control of the rate of the heart-beat predominates in the right vagus, and

control of stimulus conduction predominates in the left vagus, not because of any specific activity in the nerves themselves, but, as Dr. A. E. Cohn has suggested to us, on account of a possible difference in the anatomical distribution of the two nerves.[1] The recent work of Garrey (3) on the comparison of the action of the two vagi on the turtle's heart has added weight to the suggestion. Rothberger and Winterberg (4) have pointed out differences in the action of the two vagi in dogs similar to those we have described in man, and curves published by Hering (5) and Rihl (6) have also shown them.

In three cases, dissociation between the auricles and ventricles has occurred after stimulation of the right vagus nerve. In other words, following pressure over the right vagus, the auricular rate has been slowed and the ventricular contractions have ceased to follow normally the slowed auricular rhythm, but have established their own rhythm for one, two, or more beats. The contractions of the two parts of the heart have occurred almost synchronously, for the *P* and *R* waves nearly coincide, and the rate of the ventricles has been, at least in the first beat of the dissociations, faster than that of the auricles. The independent ventricular rates in these cases were from 57.3 to 78.5 beats per minute.

The usual cause of complete dissociation of auricles and ventricles is complete heart-block, when the normal mechanism for the conduction of the heart-beat is so impaired, usually by an anatomical lesion, that the ventricles receive no stimuli from the auricles, and so the ventricular rhythm is established independently of the auricular activity. The inherent rhythmicity of the ventricles determines then the rate of their contractions. This rate is, as a rule, in adults about thirty beats a minute, although faster ventricular rates have been observed, as in the cases of Wenckebach (7) and Windle (8), in which the ventricles beat forty-six and sixty-six times per minute respectively.

In our cases, impairment of conduction seems to play no part in the production of the dissociations, as there is but slight delay

[1] As this subject is discussed by Dr. Cohn in his paper in this number of the *Journal of Experimental Medicine*, we shall not go further into it. For the same reason, we shall not attempt to show extensively the relation of our work to the results which have been obtained experimentally on animals.

in the conduction of the heart-beat from auricles to ventricles before the dissociation, a decrease in the conduction time immediately after, and no true blockage of the heart-beat is seen. It is the inherent high rate of ventricular rhythmicity in these cases which is the determining factor in the production of the auriculo-ventricular dissociations which follow right vagus stimulation. As soon as the vagus action slows the auricular rate below that at which the ventricles would contract by their own inherent rhythmicity, the ventricles cease to wait for the auricles as their pace-maker. They break through the pause, establish their own rhythm, and interrupt the normal course of the stimuli of the heart-beat. When vagus stimulation is discontinued, the auricular rate increases to a point where it is again sufficiently rapid to resume its pace-making function. An essential feature of the action of the right vagus in the production of these dissociations is its much greater inhibitory effect on the auricular rate relative to its effect on that of the ventricles, the rate of the inherent rhythmicity of the latter being apparently uninfluenced by stimulation of the right vagus nerve.

These patients in which dissociation followed right vagus stimulation had in common an abnormally rapid heart rate when at rest; an abnormally large pulse pressure occurred at least in two of the three cases; and in all, the heart action was thumping. The foregoing facts and the form of the undisturbed electrocardiograms from these cases have led us to the conclusion that the unusually high rate of the inherent ventricular rhythm, which has been the essential causative factor in the production of the dissociations, is the result of a hypertonus of the cardiac accelerator nerves. The undisturbed electrocardiograms from the cases showed very prominent *P* and *T* waves, resembling those obtained by Rothberger and Winterberg (9), after both vagi were cut. These authors conclude that the prominence of these waves in the electrocardiograms is typical of increased cardiac accelerator activity. The *P* and *T* waves are usually well marked in children, but in our cases a further abnormal exaggeration of them is seen. It is perhaps noteworthy that the fourth case, in which dissociation never followed vagus stimulation, also failed to show any of the signs which we have taken as indicative of accelerator over-action.

The question of accelerator tone is one which has not been very generally considered and little is known as to its normal occurrence. Hunt (10), however, who investigated the question extensively in dogs, reached the conclusion that the most important function of the accelerators, and perhaps their only demonstrable one, was their tonic activity.. Hunt has shown that the accelerators not only stand in antagonism to the vagi, but that stimulation of the right accelerator has apparently more effect on the heart rate than left accelerator stimulation. Rothberger and Winterberg (4) have confirmed this finding, and they also show that left accelerator stimulation raises the power of stimulus formation in certain regions, especially in the node of Tawara and in the left auricle. This action of the left accelerator nerve on the lower points of stimulus formation could not be demonstrated, as a rule, until the activity of the normal pace-maker, the sinus node, was depressed. They found that this depression could be accomplished not only by cooling and scarification but by vagus stimulation as well. It appears from their work (9) that the right accelerator predominates over the sinus node in the right side of the heart, while the left accelerator exerts a stronger action over the heart in the region of the atrioventricular bundle. There is some reason to believe then that each accelerator antagonizes each vagus more or less separately, and that there is a certain anatomical division between the innervation of the right and left side of the heart. This is probably only relatively true, however, as the nerves of the right side go over functionally into the domain of the nerves of the left side, and *vice versa.*

Rothberger and Winterberg (4) have shown that after section of all the heart nerves in dogs, simultaneous stimulation of the right vagus and left accelerator results in the establishment of subauricular autonomy. This phenomenon depends upon the fact that the hyper-accelerated rhythmicity of the atrioventricular node exceeded the rhythmicity of the vagus-slowed auricles, and so ultimately the atrioventricular node set the pace for both auricles and ventricles. Thus a true nodal rhythm is produced. During the transition between this nodal type and the usual rhythm, there occurred a time when the sinus node set the pace for the auricles and the atrioventricular node set the pace for the ventricles. Electrocardiograms

of this phenomenon in their dogs are very similar to those we obtained from our patients during the dissociations[2] described above. In our cases, we believe that both accelerators were in a state of increased activity or hypertonus. Although the left accelerator was exerting continuously its activity on the atrioventricular node, producing a heightened state of rhythmicity, this effect did not become apparent, for the right accelerator through its action on the sinus node sustained auricular rate at such a pace that ventricular autonomy could not assert itself. When, however, the action of the right accelerator was offset by stimulation of the right vagus, the rate of stimulus formation in the sinus node was reduced momentarily below that of the highly accelerated atrioventricular node. The atrioventricular node then asserted itself as the pace-maker of the ventricles, and complete dissociation resulted.

The form of the ventricular complexes in the electrocardiograms in two cases (Nos. I and III) is the same during the dissociation as when no dissociation exists. This indicates that in spite of the fact that the stimulus of the heart-beat no longer reaches the ventricles as a result of auricular contraction, it does arise in some point above the ventricles themselves. The stimulus reaches them and passes through them in a normal manner, for it is well known that stimuli arising in abnormal points in the ventricles, as in ventricular extrasystoles, give abnormal electrocardiograms. It seems probable that the point of origin of the ventricular stimuli is the atrioventricular node of Tawara. This node is considered on account of its specialized structure to have a higher rhythmicity than other points in the region of the ventricles, and Winterberg (11) has shown that electrical stimulation of the exposed heart in this region produces a normal ventricular complex in the electrocardiogram. In the third case (No. II), the independent ventricular complex in the electrocardiogram during the dissociation has an abnormal form, and here some other point in the ventricles has taken up the rhythm.

Left vagus pressure never produced dissociation in these cases, probably, on the one hand, because stimulation of this nerve never reduced the auricular rate sufficiently to allow the ventricular au-

[2] See especially figure 4*b*, *Arch. f. d. ges. Physiol.*, 1911, cxli, 355, and figure 1*e*, *ibid.*, 1910, cxxxv, 567.

tonomy to assert itself. Possibly, on the other hand, stimulation of the left vagus directly offset the tonic action of the left accelerator and so prevented the onset of that increased ventricular rhythmicity which would find expression in dissociation. Some other factor seems to operate besides the delay of the auricular rate in allowing the ventricular autonomy to assert itself, for in the same case dissociation sometimes occurred after cardiac cycles which were shorter than some after which no dissociation took place. This added factor might be considered to be the mechanical stimulation by pressure of the accelerator fibers which so often run in the vagus nerve; the well known, long latent period of the accelerator nerves, however, makes it very unlikely that this is the case.

It is our belief that in these three cases of so-called chronic endocarditis in which right vagus stimulation produced a dissociation between auricles and ventricles, hypertonus of the accelerator nerves is a striking and important feature in the pathological physiology. It is this factor that we consider responsible for the apparent overaction of the heart, as evidenced by the rapid rate, violent contractions, and the large difference between the systolic and diastolic blood pressure, indicating an abnormally large cardiac output per beat. It is this factor also that gives these cases the somewhat distinctive form of electrocardiogram and raises the inherent rhythmicity of the ventricles to such a point that they assume their own rhythm, independently of the auricles as soon as the auricular beats are reduced to a rate which is not excessively slow. The fact that the same characteristics are seen in the electrocardiogram and that the same type of dissociation may appear when the accelerators of the dog's heart are stimulated electrically, adds weight to this idea.

It is, of course, impossible to say why the hearts of patients such as these, who suffer from chronic cardiac disease after rheumatism, should show evidence of overaction of the accelerator nerves. There may be an increased susceptibility of the heart to the action of these nerves, there may be an absolute increase in their tonic activity, or there may be a relative hypotonic condition of the vagus inhibitory mechanism which allows the usually active tone of the accelerators (if such exists) to be abnormally effectual. It is possible that this hypertonus or constant overaction of the accelerators of the heart

represents a compensatory phenomenon which has gone beyond the limits of usefulness. On account of the valvular lesions, no doubt, it is necessary for the heart muscle to increase its work per beat and per minute in order to maintain the circulation properly, but the impression is gained by clinical examinations of these patients, that their hearts beat with unnecessary violence and rapidity and that there must be a constant fatiguing strain upon the myocardium. An increase of rest for the heart muscle by slowing the rate of its contractions is certainly an essential factor in restoring the compensation in many cases of heart disease. Besides rest in bed and such general measures as ice to the praecordium, there is often nothing of avail in reducing the heart rate, for in many cases digitalis and its allies fail, as do all other drugs and procedures, to produce any permanent benefit. If hypertonus of the accelerators is an important feature in the pathological physiology of these cases, as we believe it is, a rational therapy should be directed at lowering the activity of the cardiac accelerator nerves. This may require a new form of cardiac therapeutics.

SUMMARY.

The electrocardiographic records taken during vagus stimulation by pressure from children suffering with chronic heart disease have shown that the stimulation of the vagi in these cases is strikingly effectual. In some of the cases, a definite difference was demonstrated between the action of the right and left vagi. The control of the rate of the heart-beat seemed to predominate usually in the right vagus nerve, while the control of stimulus conduction from auricles to ventricles apparently predominated usually in the left vagus. This difference in the two nerves probably exists on account of the difference in their anatomical distribution, the right vagus going especially to that part of the heart which controls the rate of contraction, the sinus node above the right auricle, and the left vagus going especially to that part in which the conducting mechanism is found. Each nerve, however, has to a lesser degree the function which predominates in the other. The whole heart seems to respond, as a rule, more promptly to right than to left vagus pressure, and fairly constant differences have been seen in the effects which stimu-

lation of each nerve has on the various waves of the electrocardiograms. The stimulation of each vagus may influence directly the contractions of the ventricles, causing great diminution in their force.

Right vagus stimulation was followed at times by a complete dissociation of auricles and ventricles. The auricular rhythm was slowed sufficiently at this time to allow the ventricles, whose inherent rhythmicity is apparently unaffected by right vagus stimulation, to take up their own independent rhythm. The heightened ventricular rhythmicity in these cases allowed this to take place after only moderate slowing of the auricles. The independent ventricular rhythm was sometimes established in the region of the node of Tawara, for no disturbance of the ventricular portion of the electrocardiogram occurred. At another time, some other point in the ventricles inaugurated the stimuli of the independent ventricular contractions and an abnormal electrocardiogram resulted.

The resemblance of our curves, showing dissociation, to those obtained during right vagus and left accelerator stimulation in dogs is definite. That analogy, the clinical picture, and the form of the electrocardiograms of these cases have led us to the belief that an important feature in the pathological physiology of these cases is hypertonus of the cardiac accelerator nerves. This factor, as a cause of symptoms and as a hindrance to the establishment of cardiac rest, may prove of great importance, against which a new form of cardiac therapeutics must be directed.

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EXPLANATION OF PLATES.

Plate 1.

Fig. 1. Curve 19.4. Case I. Without vagus pressure.

Fig. 2. Curve 27.1. Case I. Without vagus pressure.

Fig. 3. Curve 27.2. Case I. Left vagus pressure.

Fig. 4. Curve 19.2. Case I. Right vagus pressure with dissociation of auricles and ventricles.

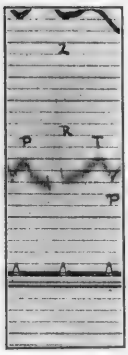


FIG. 1.

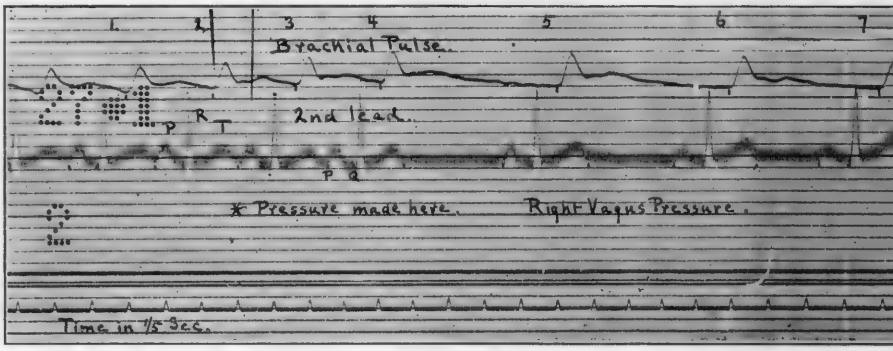


FIG. 2.

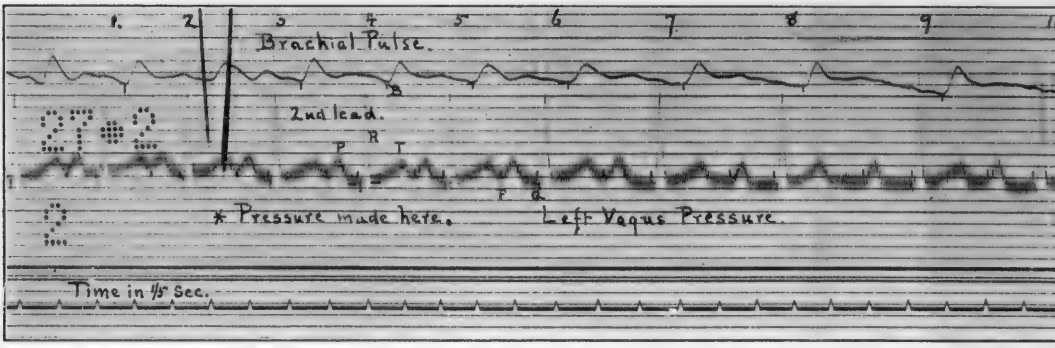
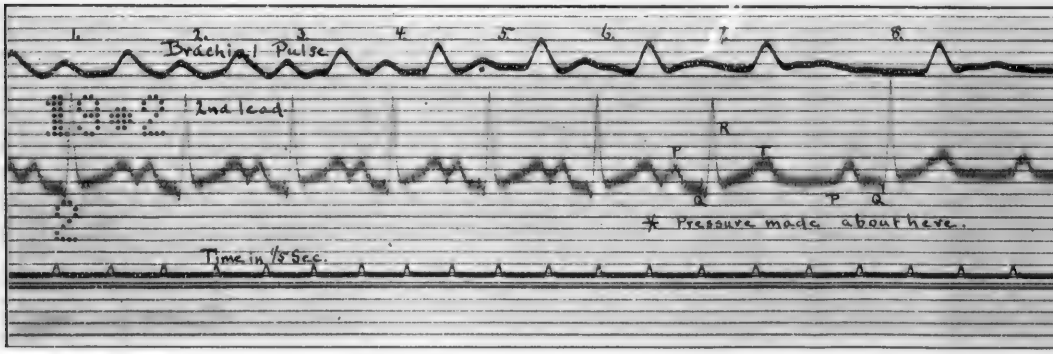
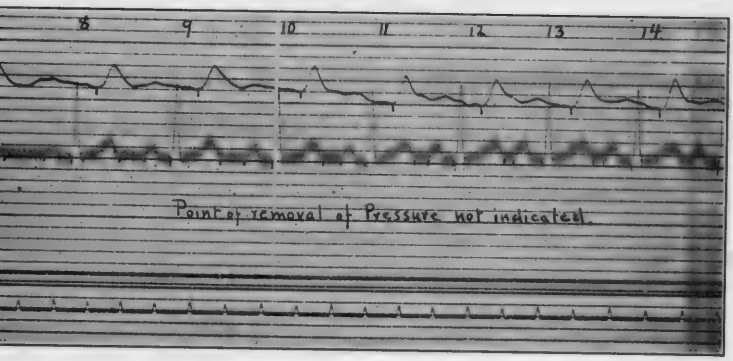
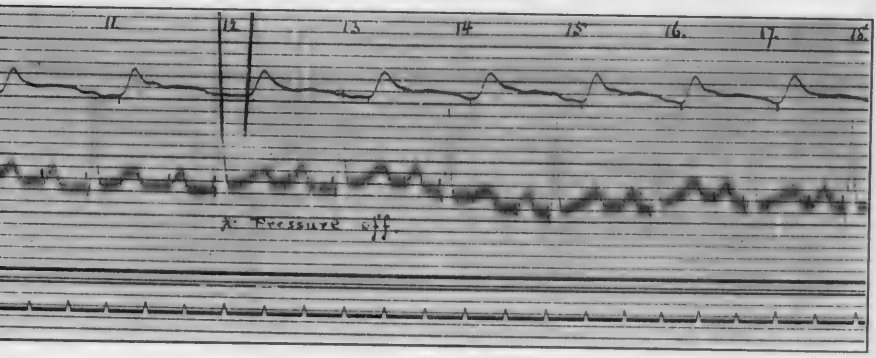


FIG. 3.




8
9
10
11
12
13
14
Point of removal of Pressure not indicated.


11
12
13
14
15
16.
17.
18.
At Pressure off.

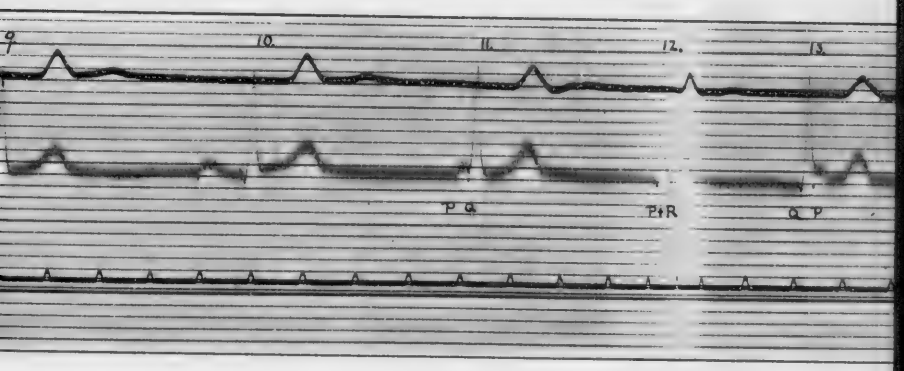
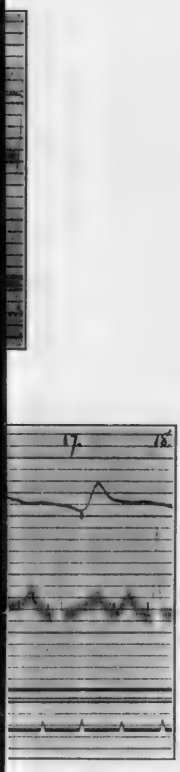

9
10.
11.
12.
13.
P Q
P R
Q P

FIG. 4.

PLATE 1.


17.
18.

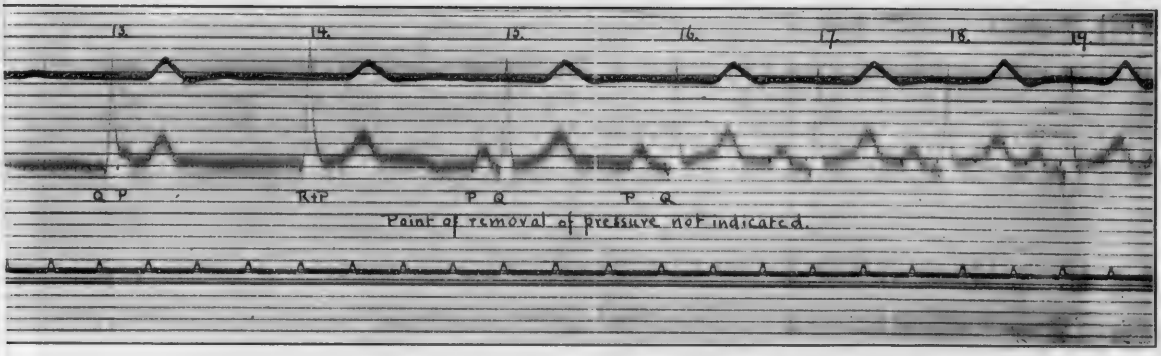

13.
14.
15.
16.
17.
18.
19.
Q P
R P
P Q
P Q
Point of removal of pressure not indicated.

PLATE 2.

FIG. 5. Curve 19.5. Case I. Right vagus pressure. Cardiogram and electrocardiogram.

FIG. 6. Case I. Graphic record of the apex beat and brachial artery during right vagus pressure.

FIG. 7. Curve 41.4. Case II. Without vagus pressure.

FIG. 8. Curve 41.2. Case II. Right vagus pressure.

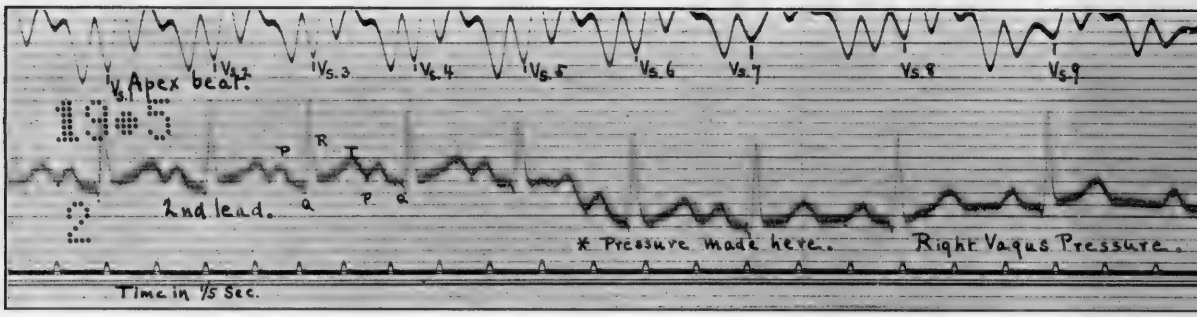

Vs Apex beat.
Vs.2
Vs.3
Vs.4
Vs.5
Vs.6
Vs.7
Vs.8
Vs.9
P
R
T
2nd lead.
Q
P
Q
* Pressure made here.
Right Vagus Pressure.
Time in 1/5 Sec.

FIG. 5.

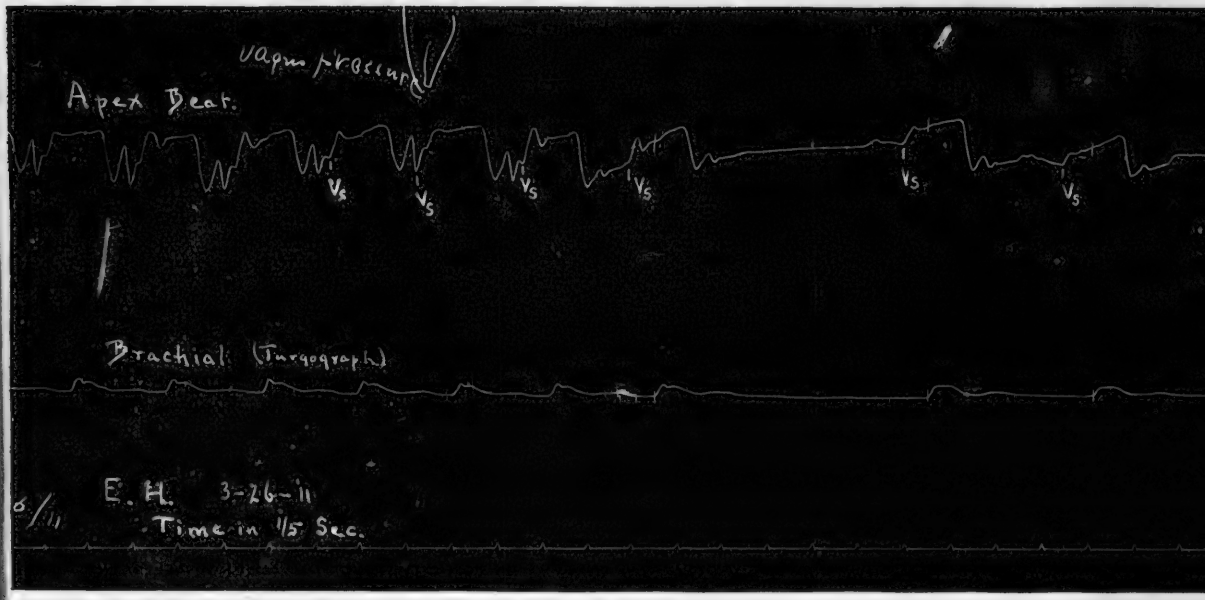
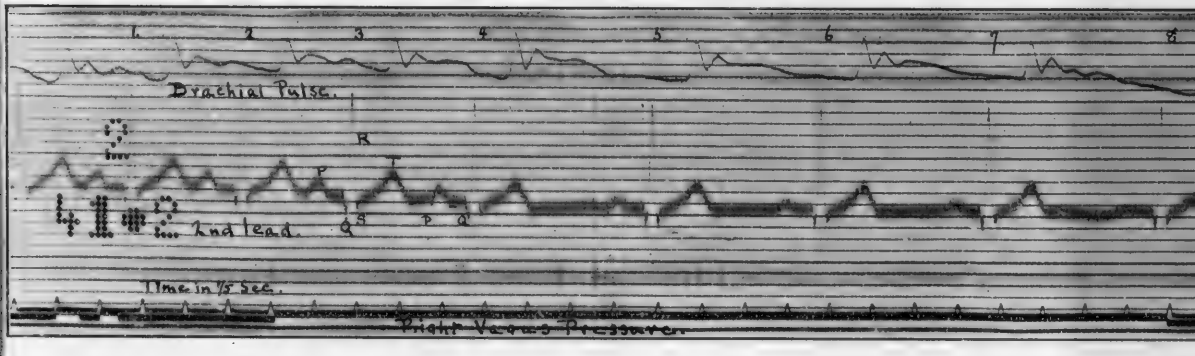
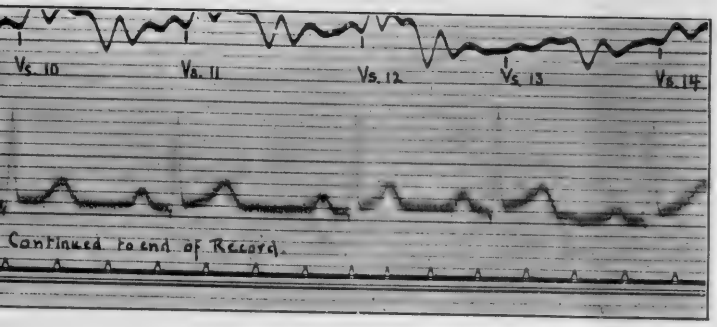

Vagus pressure
Apex Beat.
Vs
Vs
Vs
Vs
Vs
Vs
Brachial (Tangograph)
E. H. 3-26-11
Time in 1/5 Sec.

FIG. 6.


1
2
3
4
5
6
7
8
Brachial Pulse.
R
P
T
2nd lead.
Q
S
P
Q
Time in 1/5 Sec.
Right Vagus Pressure.

FI


Vs. 10
Vs. 11
Vs. 12
Vs. 13
Vs. 14
Continued to end of Record.

Vs

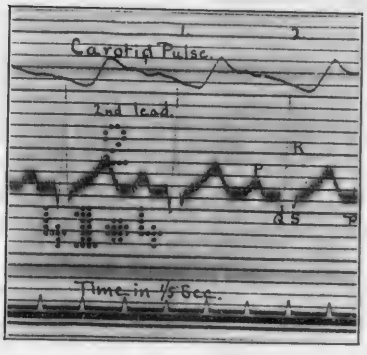
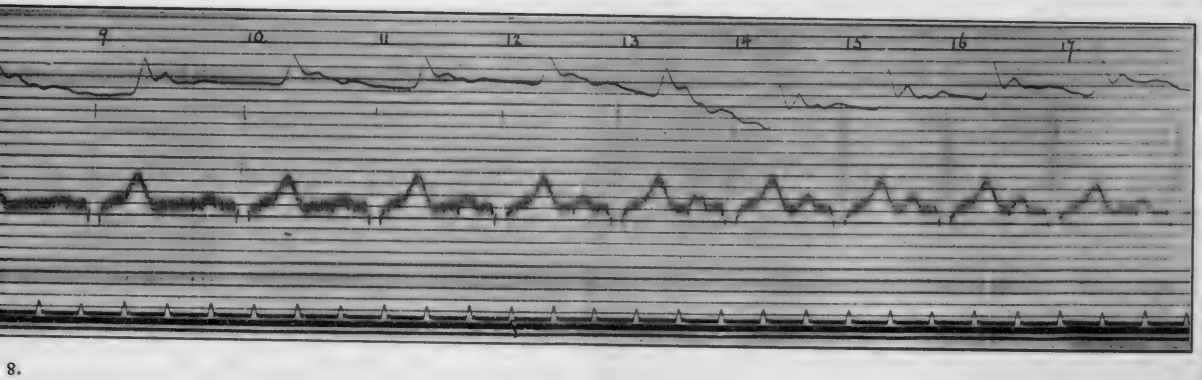

Carotid Pulse.
2nd lead.
R
S
T
Time in 1/5 Sec.

FIG. 7.


9
10
11
12
13
14
15
16
17

8.

PLATE 3.

FIG. 9. Curve 41.6. Case II. Left vagus pressure.

FIG. 10. Curve 41.3. Case II. Right vagus pressure with dissociation of auricles and ventricles.

FIG. 11. Curve 47.1. Case III. Without vagus pressure.

FIG. 12. Curve 66.2. Case III. Right vagus pressure with dissociation of auricles and ventricles.

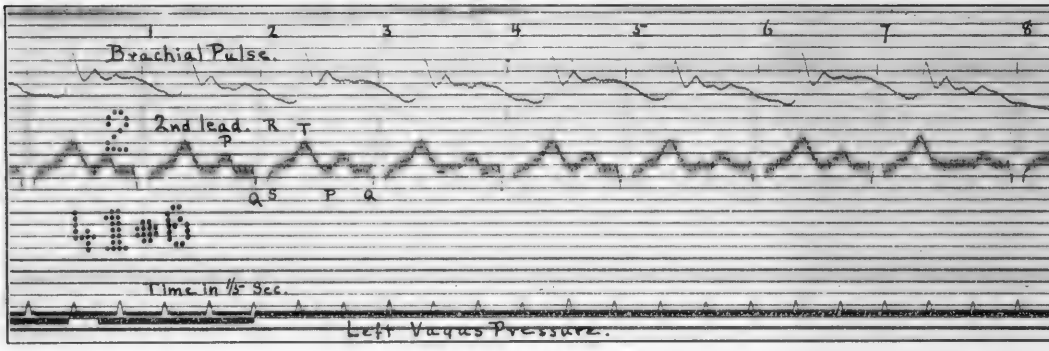
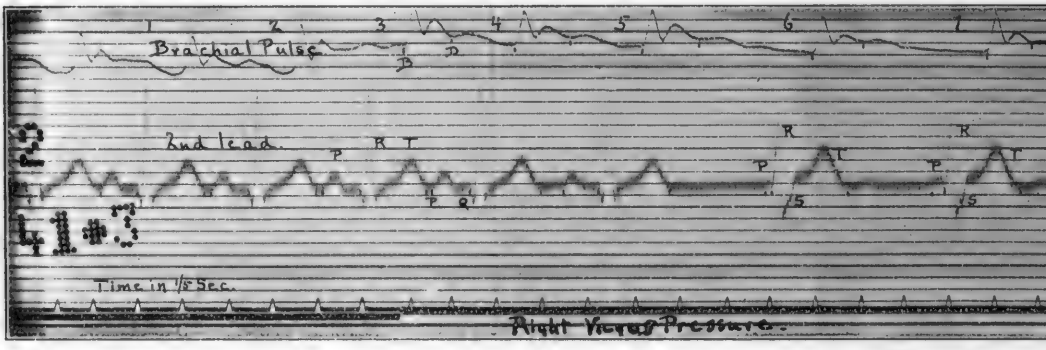
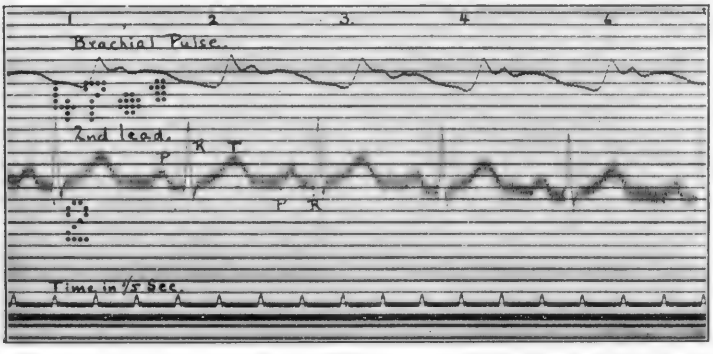

1
2
3
4
5
6
7
8
Brachial Pulse.
2nd lead.
R
T
P
Q
S
P
Q
Time in 1/5 Sec.
Left Vagus Pressure.

FIG. 9.


1
2
3
4
5
6
7
Brachial Pulse.
2nd lead.
P
R
T
P
Q
R
T
P
S
R
T
P
S
Time in 1/5 Sec.
Right Vagus Pressure.


1
2
3.
4
6
Brachial Pulse.
2nd lead.
R
T
P
P
R
Time in 1/5 Sec.

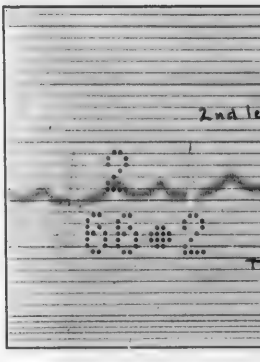
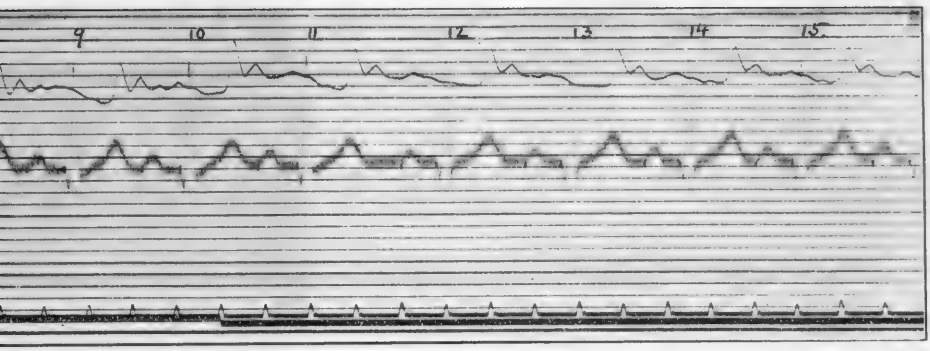

2nd le
T

FIG. 11.


9
10
11
12
13
14
15

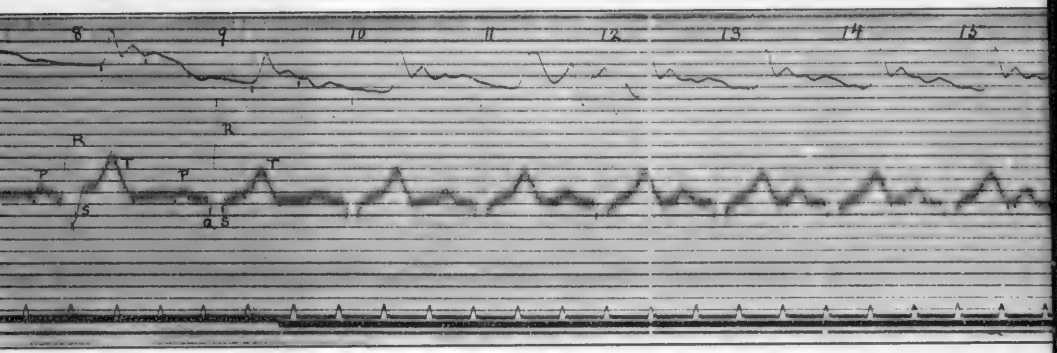

8
9
10
11
12
13
14
15
R
R
T
T
P
T
S
Q S

FIG. 10.

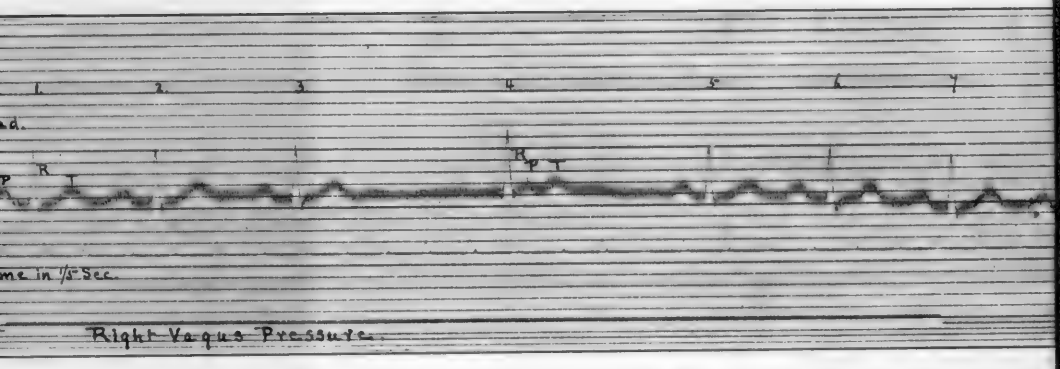
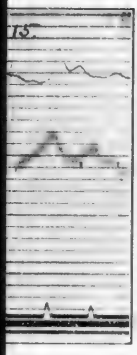
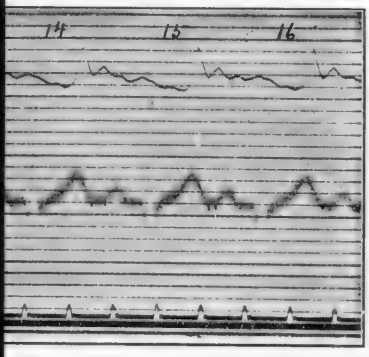

1
2
3
4
5
6
7
R
T
R
P
T
me in 1/5 Sec.
Right Vagus Pressure.

FIG. 12.

PLATE 3





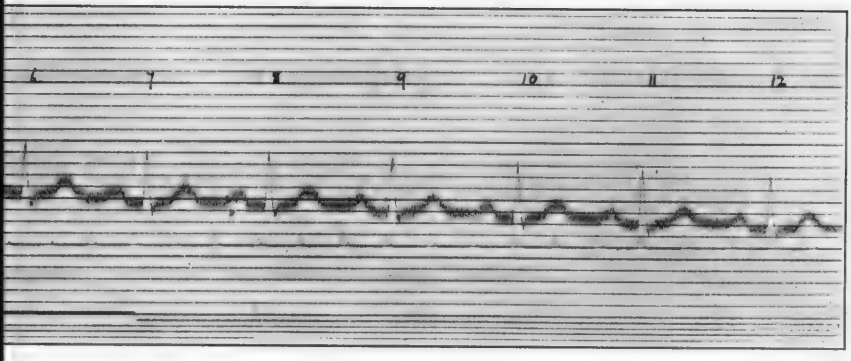


PLATE 4.

FIG. 13. Curve 66.4. Case III. Left vagus pressure.

FIG. 14. Curve 77.5. Case III. Right vagus pressure showing dissociation and the effect on the cardiogram.

FIG. 15. Curve 77.4. Case III. Left vagus pressure. Cardiogram and electrocardiogram.

FIG. 16. Curve 38.3. Case IV. Without vagus pressure.

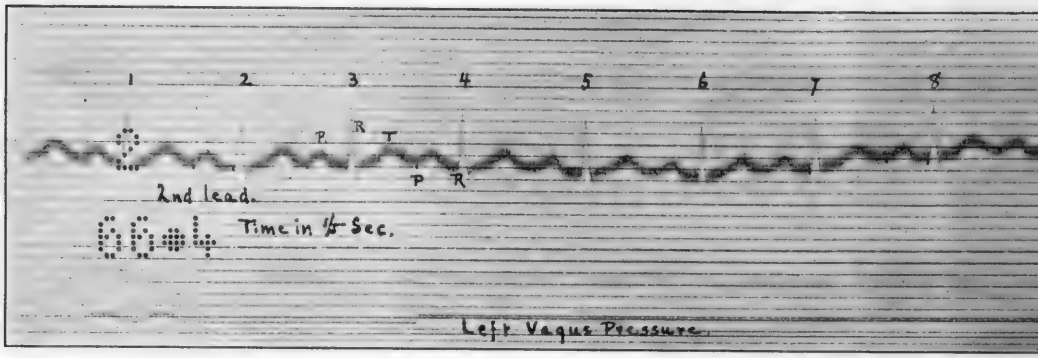
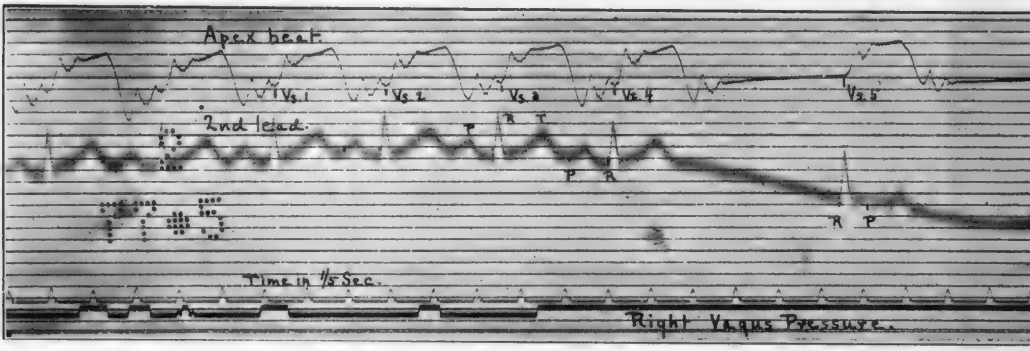

1
2
3
4
5
6
7
8
P
R
T
P
R
2nd lead.
Time in 1/5 Sec.
Left Vagus Pressure.

FIG. 13.


Apex beat.
Vs. 1
Vs. 2
Vs. 3
Vs. 4
Vs. 5
2nd lead.
P
R
T
P
R
R
P
Time in 1/5 Sec.
Right Vagus Pressure.

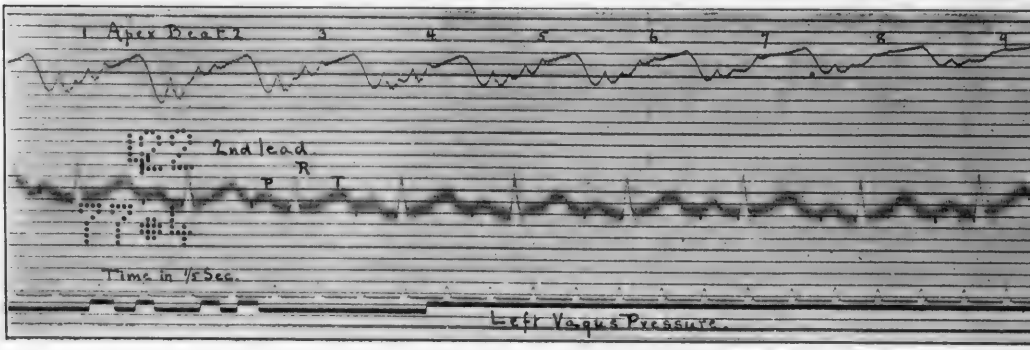

1 Apex Beat 2
3
4
5
6
7
8
9
2nd lead.
R
P
T
Time in 1/5 Sec.
Left Vagus Pressure.

FIG. 15.

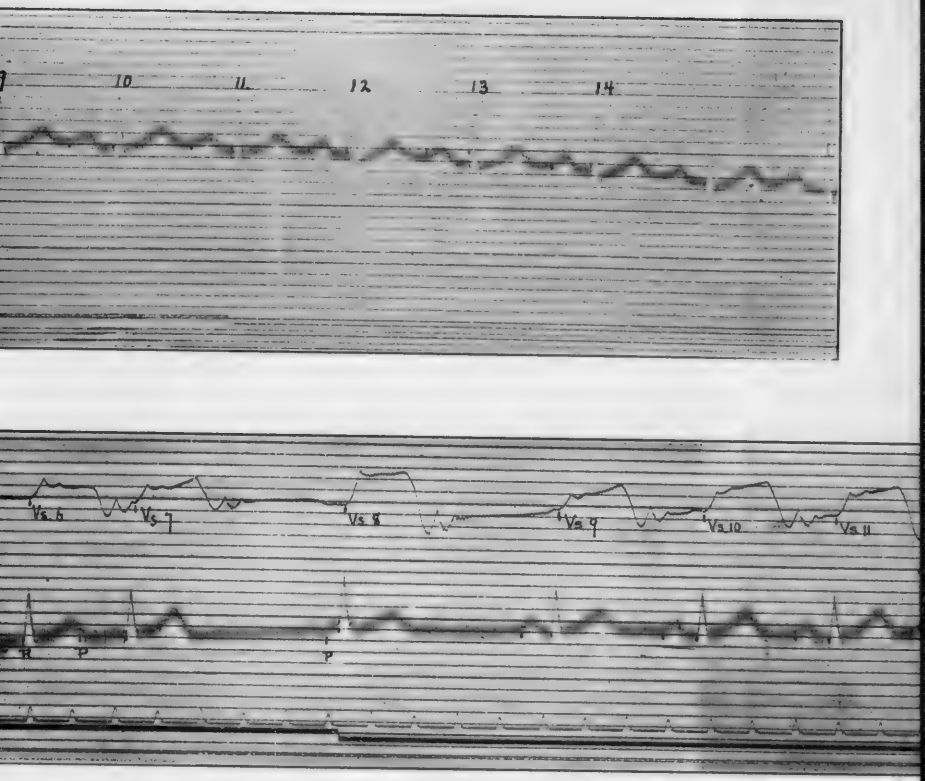
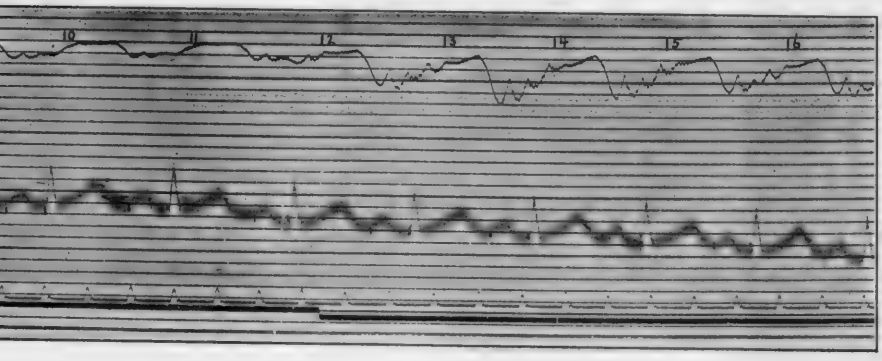


FIG. 14.



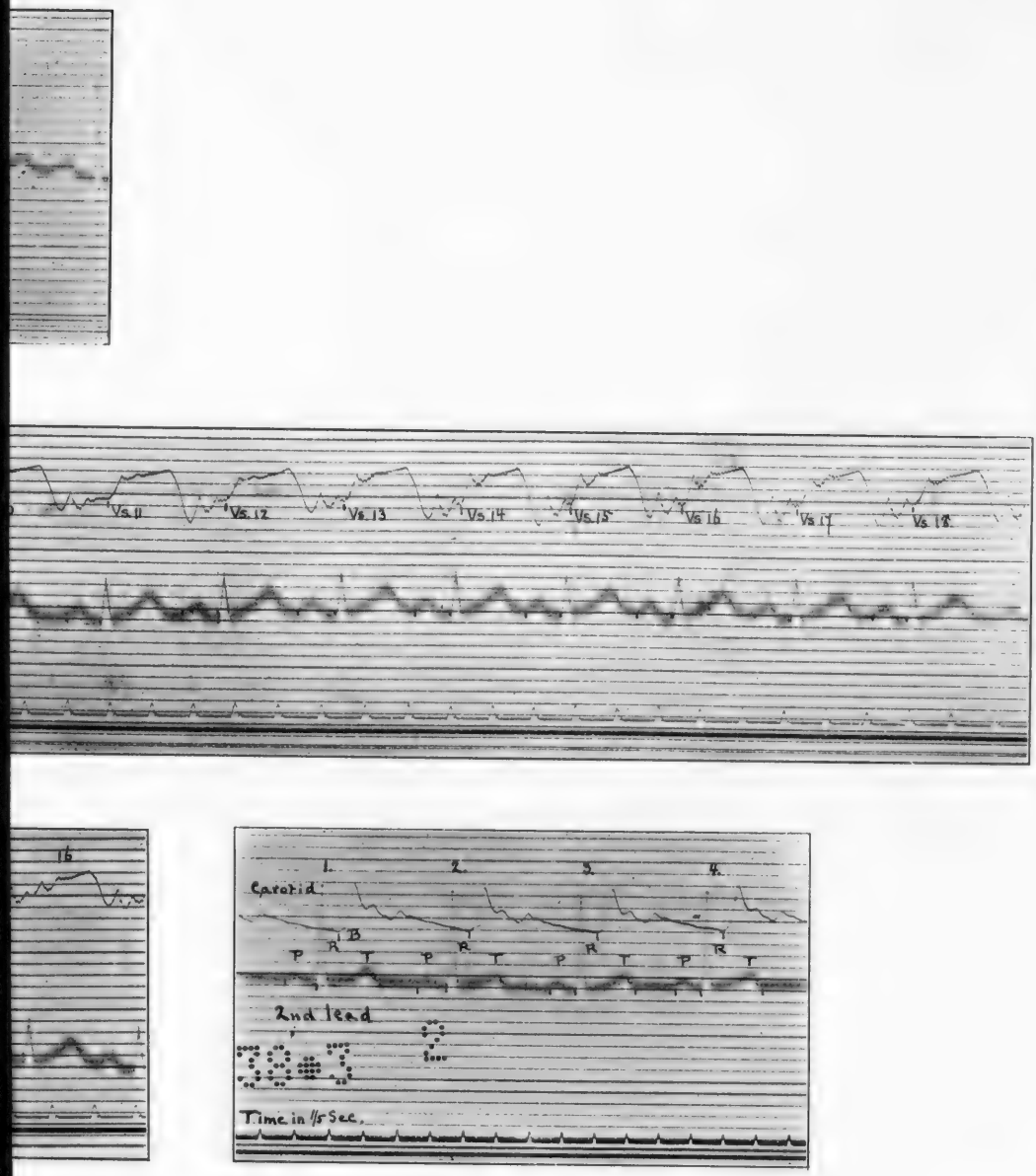

Vs 11
Vs 12
Vs 13
Vs 14
Vs 15
Vs 16
Vs 17
Vs 18
1b
1.
2.
3.
4.
Carotid.
P
R
T
2nd lead.
Time in 1/5 Sec.

FIG. 16.

PLATE 5.

FIG. 17. Curve 38.4. Case IV. Right vagus pressure.

FIG. 18. Curve 38.5. Case IV. Left vagus pressure.

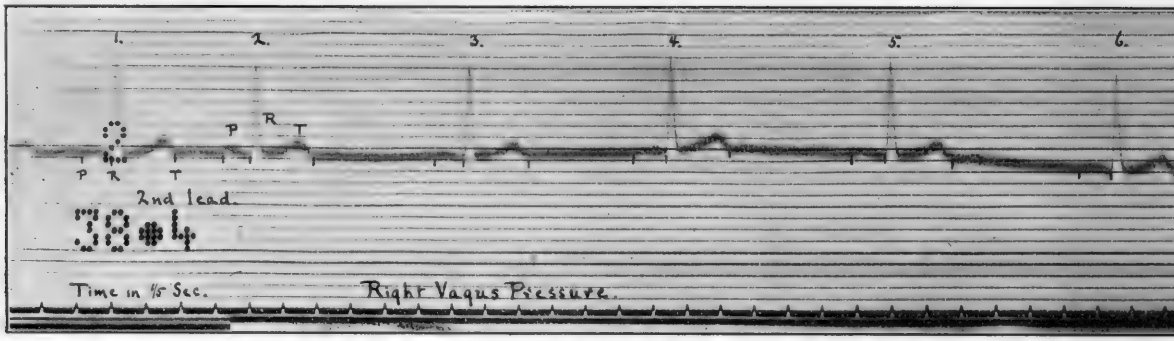

1.
2.
3.
4.
5.
6.
P
R
T
2nd lead.
Time in 1/5 Sec.
Right Vagus Pressure.

FIG.

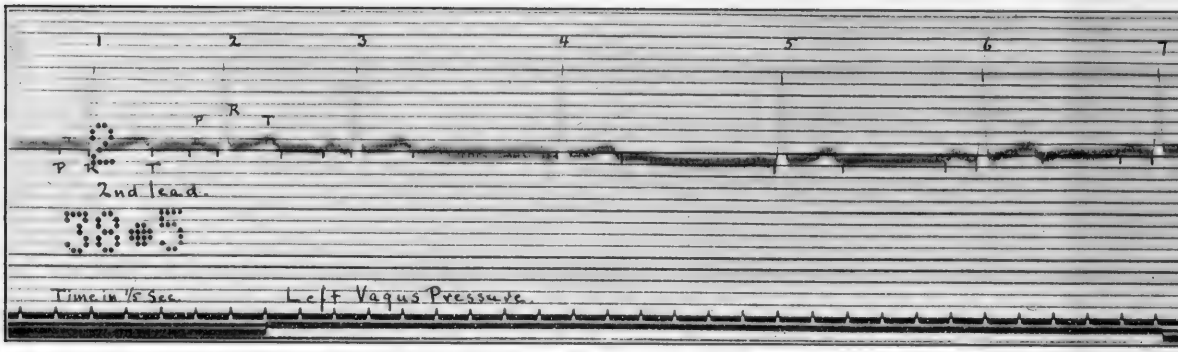
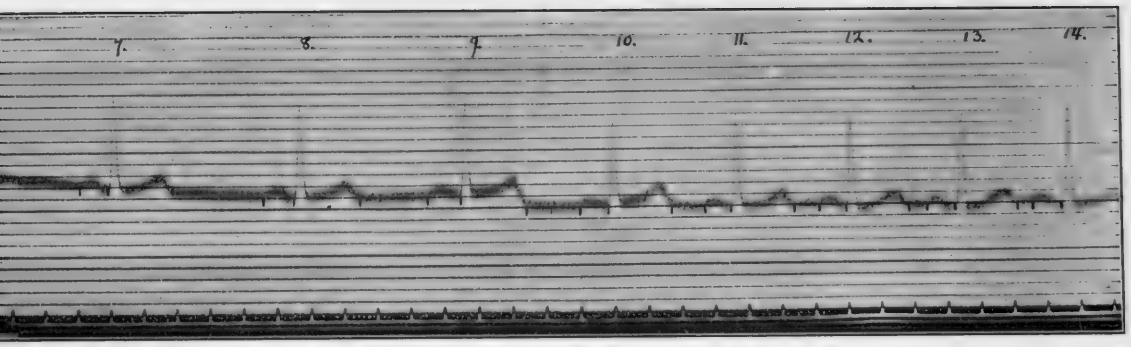
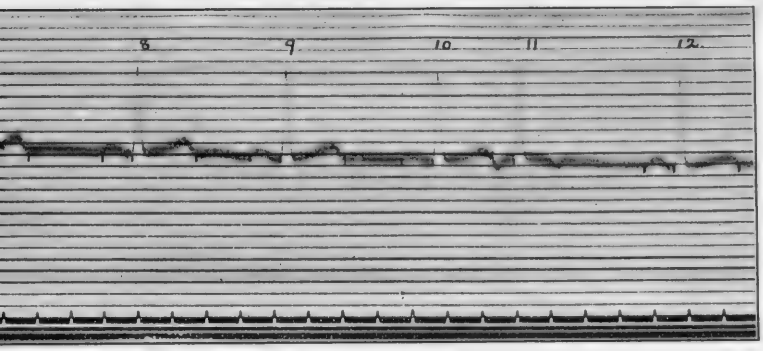

1
2
3
4
5
6
7
P
R
T
2nd lead.
Time in 1/5 Sec.
Left Vagus Pressure.

FIG. 18.

PLATE 5.





AURICULAR TACHYCARDIA WITH A CONSIDERATION OF CERTAIN DIFFERENCES BETWEEN THE TWO VAGI.*

By ALFRED E. COHN.

(*From the Department of Pathology of the College of Physicians and Surgeons, Columbia University, New York.*)

PLATE 6.

The experiments reported in this paper were designed to explain the abnormalities observed in the following cases of auricular tachycardia: (1) a case described in 1908 by Hertz and Goodhart (1), (2) three cases published recently, in 1911, by Rihl (2), and (3) one,[1] still unpublished, which the author observed this year (1910–1911).

Although all the cases had points of close similarity, the most essential characteristic was the high auricular rate. In the first case, that of Hertz and Goodhart, the highest rate noted was 236. This was supposed by the authors to represent the speed limit of the heart. In the cases reported by Rihl, the auricular rate in the first case was about 300, that in the second 200, and that in the third 200. In the author's unpublished case, the rate was 280, there being but slight variations from time to time. It is extraordinary that the auricular rate in these five cases should be so unusually high, and it is more remarkable still that in all of them the rate should be maintained with but very slight fluctuation for weeks and months—in fact, throughout the entire period of observation. Under ordinary circumstances, this unusual condition might escape observation, for the ventricular rate in these cases was either normal or, as in Hertz and Goodhart's case, slightly above normal. In Rihl's first case, the radial rate at first examination was 150, but three weeks later it had fallen to 70, which seems to have been the usual speed; in the second case, the rate on admission was 80, and twenty months later it was 72. In this case, the radial rate sometimes rose from 100 to 150, as a result of muscular exertion and excitement, and went even as high as 200. In Rihl's third case, the radial rate was about 100; once it rose to 200. In the author's unpublished case, the usual rate was 70, and, except following some interference, such as the administration of atropin, it remained at this speed. It is apparent, therefore, that unless a special examination had been made, the

* Received for publication, November 8, 1911.

[1] This patient was in the service of Dr. Manges, 2d Division, Mount Sinai Hospital.

unusual auricular condition would not have been suspected. It may be stated that unless affected by special conditions, all these hearts showed a definite auriculo-ventricular coördination. Most frequently the ventricle responded to every fourth auricular beat, that is, a ratio of 4 to 1. At such times the ventricular rate was regular. Frequently, however, the ventricular rhythm appeared disordered, and analysis (especially in Rihl's cases) indicated that the ventricles were then responding to every third or to every second auricular contraction.

Rihl tested the effect of vagus pressure (Czermak) in his cases, and in all of them marked slowing or stoppage of the ventricles occurred. He usually fails to state whether pressure was made on the right or the left vagus. In one instance, however, he mentions that the pressure was on the right vagus, and also states that in this case similar results were obtained by pressing on the left side. Curves of the effect of pressure on the left side, however, are not reproduced. While vagus pressure in these cases induced the usual ventricular slowing, absolutely no effect on the auricular rate could be detected. In all five cases the effect of atropin on the heart rate was studied. In each instance, the ventricular rate was markedly increased, while the auricular rate remained unaltered. Finally, in Rihl's three cases, digitalis decreased the number of ventricular systoles; that is, it increased the degree of heart-block between the auricles and ventricles, while at the same time the auricular rate again remained unaffected. In these cases, therefore, by increasing vagus irritability (pressure and digitalis) the ventricular rate was decreased, and by decreasing vagus irritability (elimination of vagus control by atropin), the ventricular rate was increased. The auricular rate, however, remained uninfluenced by these procedures. In other words, the auricles in these cases have escaped chronotropically from vagus control.

Rihl[2] has assumed that this emancipation of the auricles is due, on the one hand, to absent vagus control and, on the other, to increased accelerator influence. It is difficult to accept this theory, because if the disturbance were in the peripheral nerves, then a continued or an increased control of the ventricles by the nerves, which he also assumes,[3] is hardly reconcilable with a complete loss of control of the auricles.

In explanation of the phenomena in these cases, one might assume that the site of stimulus production has become anatomically dislocated from the sino-auricular node, and is now beyond the reach of the rate-controlling vagus. In one of Rihl's cases, such a dislocation of the site of stimulus production is indicated by the diminished *A–C* interval and also by the appearance of a negative *P* wave in curves from the second lead. However, numerous curves of the author's unpublished case show a positive *P* wave, wherefore stimulus production must have been at or near the usual site, and to this, of course, vagus influence could reach. A peripherally active vagus and a normally placed stimulus-producing area are therefore known

[2] Rihl, *loc. cit.*, p. 310.
[3] Rihl, *loc. cit.*, p. 311.

to exist in this case. In view of the fact that we know that the vagus reached and influenced the ventricles, the lesion must be looked for in the fibers of the vagus to the auricle, or in the vago-auricular junction, or in the auricle, or possibly in the sino-auricular node itself. A consideration of accelerator action will not be discussed here.

When the author's case came under observation,[4] it occurred to him that differences in the action of the right and left vagus might account for the findings recorded. The demonstration of such differences in the action of the two vagi would be an important step toward an explanation of the mechanism of these cases. The following experiments were therefore made, with the view of ascertaining whether or not there were differences in the action of the right and left vagus on various portions of the heart.

The experiments were carried out on the hearts of dogs, perfused *in situ*. The two vagus nerves were dissected out. The right auricle and the left ventricle were attached to receiving tambours which, in turn, communicated by air transmission with two recording tambours. The recording was done in ink on a white paper kymograph. The right and left vagus nerves were stimulated with a faradic current of varying strength. In some of the experiments, ventricular fibrillation had already occurred spontaneously, or had been induced. Whether the ventricles were fibrillating or not, differences in the action of the two vagi on the auricles could be made out. This is illustrated by the curves of experiment 587. Stimulation of the right vagus (figure 12) in the neck produced no effect, probably because excessive injury had been done to the nerve in dissection. Stimulation of the right vagus in the chest (figure 13), however, caused complete inhibition of auricular movement. Stimulation of the left vagus in the neck (figure 11), on the other hand, produced no chronotropic change whatever, though a marked negative inotropic change is clear. In another experiment (No. 581), stimulation of the right vagus (figure 2) caused only a marked negative inotropic change, while stimulation of the left vagus (figure 1) was without effect. In these two experiments there was ventricular fibrillation, and vagus stimulation (right or left) failed

[4] In December, 1910, before the publication of Rihl's cases.

to relieve this condition and to restore the ventricles to orderly contraction. Here is an example of an auricle chronotropically uninfluenced by either right or left vagus stimulation. This is a condition comparable to that found in the cases reported. In this experiment (No. 581), the sino-auricular node had been excised, and it is possible that the clinical phenomena may be associated with a comparable event. This is the only experiment in which the right vagus was chronotropically ineffectual.

Differences in the action of the two vagi were also observed in the absence of ventricular fibrillation. In experiment 584 (figure 3), both vagi were stimulated at 0. The right vagus stimulation slowed the whole heart, while the left vagus stimulation was ineffectual. Stimulation of the right vagus in experiment 586 (figure 9) stopped both auricles and ventricles. The effect on the ventricles, however, was not profound, as is seen by the slight negative inotropic effect, whereas the effect on the auricles was most marked and far outlasted even the slight diminution in the size of the ventricular contractions. Stimulation of the left vagus (figure 8) with a current of the same strength was ineffectual in producing any effect, but with a somewhat stronger current (figure 10) the ventricular contractions were entirely arrested. The slight movements seen in the ventricular curve were conveyed to the pen by the signal. Another error in this curve is due to the fact that the signal records a period of vagus stimulation, after the electrodes had been removed from the nerve. At the same time, however, it can be seen clearly that auricular movements did not come to an end, although their strength was greatly reduced.

A final illustration of the difference between the action of the two nerves is given in the curves from experiment 585. Stimulation of the right vagus (figures 4 and 6) results in complete stoppage of the whole heart, the ventricles following the auricles. Stimulation of the left vagus (figure 5), however, is followed by ventricular stoppage only. The auricle continues to beat during this period, but at a reduced rate and with a somewhat irregular rhythm. The auricular contractions have, in fact, the appearance of breaking through the vagus inhibition. Somewhat similar events are seen in figure 7, where again the auricles escape from the vagus control,

but here, unlike the events shown in figure 5, the auricular contraction is followed by three ventricular contractions. During the remainder of the period of vagus faradization, the ventricles remain asystolic, although the auricles continue to beat. In these four curves of experiment 585, negative inotropic effect is greater on the auricles than on the ventricles, whether it is the right or the left vagus that is stimulated.

The inference to be drawn from these curves may be summed up as follows: (1) the auricles are always affected by right vagus stimulation; the ventricles may also be affected, either primarily or secondarily; (2) the ventricles are controlled by left vagus stimulation; the auricles may or may not be affected. The curves here presented are selected from a large number. All the experiments did not show such differences as are here demonstrated. There is a definite lack of uniformity in the results obtained, but where differences in the action of the two nerves were demonstrable, it was always in the direction indicated. A discussion of the significance of these results is given at the end of this communication. It may be stated here, however, that it is appreciated that for the present purposes, the method of perfusion is open to objection, and we should hesitate to present these results, were it not for the close similarity between them and others obtained from the study of normal hearts. Further experiments dealing with this subject are still in progress.

An examination of curves from experiments found in the literature shows, in the first place, that in studying the effect of vagus stimulation on the heart, little attention has been paid to the vagus stimulated. However, experiments have been reported in which both vagi have been employed and in which differences in the effect of the stimulation of the two vagi have been mentioned by the authors or shown in their curves. The predisposition to regard the function of the two vagi as identical has usually led the writers to attempt to explain the differences in the effect of left nerve stimulation from that obtained by right nerve stimulation as due to defects in technique. These differences, however, are of sufficient importance to direct attention to them, with the view of interesting other workers in this field.

A review of all the experiments that have been performed on the cardiac nerves, especially the vagi, is unnecessary. It is useful, however, to collect from the large number of experiments already performed by others, those that relate to the present theme. Erlanger (4), Hering (5), Rihl (7), Rothberger and Winterberg (8), Flack (12), Garrey (11), Jürgens (10), and Muhm (9) have all studied the action of the extrinsic nerves of the heart.

Differences in the action of the two vagus nerves have been discovered and studied under two conditions: (1) In one set of experiments there has been no interference with the normal mechanism of the heart, except that necessary for the purposes of registration. These are illustrated by the experiments already detailed. (2) In other experiments, interference with the stimulus-producing or conducting mechanism of the heart has been attempted. In this group are included the heart-block experiments and those in which the sinus node has been attacked. We shall first consider those in which heart-block has been induced.

Erlanger (4), Hering (5), and Rihl (7) have tested the vagi in a number of such cases and have arrived at somewhat different conclusions in regard to what actually takes place. Hering and Rihl say that stimulation of the vagi inhibits the rate of the ventricles, though it may do so very slightly, while Erlanger denies that such stimulation alters the ventricular rate at all. If the cases of complete clinical heart-block in which atropin was used may be drawn upon for deciding evidence, Erlanger's contention must be accepted, for in these cases atropin never induces a relief of the supposed vagus inhibitory control. We do not know, however, whether all the vagus fibers travel to the ventricles by the path of the *A-V* bundle. Hering[5] thinks that they do not. Conclusions based on experiments made with the supposition that the ventricles are necessarily relieved of vagus control by a transverse lesion of the bundle, may be in error, as will be seen. If the vagi do not pass along with the bundle, no final conclusions can be drawn from experimental cases of heart-block, while if only one, or parts of one, or parts of both, pass, then experiments made after transverse lesions of the bundle must be interpreted with these possibilities in mind. We cannot see that Hering, Rihl, or Erlanger has considered in sufficient detail these anatomical possibilities, which would modify considerably any conclusion concerning vago-ventricular physiology. It must also be borne in mind that the paths and endings of the right and left vagus may differ. No such distinction as this was considered by Erlanger or by Hering.

Differences in the effect of stimulation of the right and the left vagus can be demonstrated when there is auriculo-ventricular dissociation. In a curarized rabbit, Hering[6] produced auriculo-ventricular block by tying and crushing the *A-V* bundle; auriculo-ventricular dissociation took place. When he stimulated the right vagus, the auricles were inhibited to the point of stoppage; the ventricles also were slowed, but continued to beat during the entire auricular asystole.[7] When he stimulated the left vagus, however, cessation of the ventricular beat occurred after a time; this was followed by a period of ventricular slowing without any effect whatever on the auricles.[8] The curves from this experiment are comparable to the curves of another experiment of Hering's to be mentioned later, where, in the absence of *A-V* dissociation, stimulation of the right and left vagus produced effects similar to these. That is to say, stimulation of the right vagus produced a primary effect on the auricles, and either none or only a slight one on the ventricles, while stimulation of the left vagus produced slowing or stoppage of the ventricles without any effect on the auricles.

[5] Hering, *loc. cit.*, p. 288.
[6] Hering, *loc. cit.*, p. 288, curves 15 and 16.
[7] Hering, *loc. cit.*, curves 11 and 15.
[8] Hering, *loc. cit.*, curve 16.

In Rihl's (7) experiments, the right vagus imperfectly controlled the ventricles (as shown in figure 1 of his report), so that in this case they were controlled apparently independently of the auricles, the auricles being asystolic; where the *A-V* groove was clamped, stimulation of the left vagus affected both auricles and ventricles, the auricles more than the ventricles (figure 2); but in another experiment (figure 4), where the left vagus was again stimulated, the ventricles alone were affected. Although we are told that in this experiment the auricles did not beat, the question arises as to what the waves between the waves of ventricular contraction signify. From their rate, one judges that they may possibly be of auricular origin. If that is the case, the left vagus in this instance inhibited the ventricles only. Other curves (Nos. 5, 6, and 7) show the effect of right vagus stimulation when no *A-V* block was present. In this case, the auricles stopped before the ventricles, but the ventricles did not escape from the inhibitory control before they should.

Erlanger has, unfortunately, not designated which vagus nerve he stimulated in his heart-block experiments. Occasionally he refers to the right nerve. In the absence of data on the action of the left nerve in his experiments, any comparison between his results and those of other authors is out of the question.

Stimulation of the right vagus never induces inhibition of the ventricles without inhibition of the auricles, when heart-block is not present. When the auricles are inhibited, the ventricles invariably follow them, although at times not so perfectly as at others. Now it is by no means proved that this ventricular inhibition is due to the fact that the ventricles depend upon the auricles for stimulation. If, on the other hand, stimulation of the left vagus, in the absence of a transverse lesion of the *A-V* bundle, results in inhibiting the ventricles alone, while the auricles continue at the old pace, we are justified in speaking of a pure ventricular inhibition. Curves illustrating this independent ventricular control are shown in Hering's (6) paper in curves 5, 6, 7, and 8. Stimulation of the right vagus in these cases caused slowing of the whole heart—auricles and ventricles (see Hering's curves, Nos. 1, 2, 3, and 4).

Garrey (11) has also shown that in the tortoise heart stimulation of the left vagus fails to control the auricles.

Rothberger and Winterberg (8) have obtained and recognized differences in the action of the two vagi. In a recent paper, they have described an experiment[9] which shows differences in the action of the right and left vagi on the auricles and ventricles, similar to those shown by Hering's curves. Stimulation of the right vagus caused complete stoppage of the auricles, while stimulation of the left vagus merely slowed the auricles, though the ventricles were entirely inhibited. They have also reproduced one curve (7*b*) which shows that stimulation of the left vagus may completely inhibit the ventricles, while the auricles continue to beat in an unaltered rhythm. The synchronous stimulation of the left accelerator probably had no influence on the result of the experiment from our point of view. Although they have recognized this difference in the action of the two vagi, they lay no particular stress upon it. Unfortunately, they studied the effect of stimulation of the right and left nerves in detail in this experiment only. They explain this phenomenon by saying, "Ausnahmsweise aber verlaufen auch die hemmenden Fasern für den Sinus Knoten fast ausschliesslich im rechten, für die Atrioventrikulargrenze dagegen vorzugsweise im linken Vagus."

[9] Rothberger and Winterberg, *loc. cit.*, p. 356.

In Muhm's (9) paper, there is a curve (table VI, No. 5) in which, on vagus stimulation, the ventricles ceased beating, while the auricles continued to contract practically unchanged. Unfortunately there is no statement as to which vagus was stimulated, so that the experiment cannot be classified. It is noteworthy that, on stimulating the vagus, ventricular asystole occurred in the presence of auricular contractions at an unchanged rate. As will be seen later, it seems likely that, just as one can now distinguish from its electrical complex whether an extrasystole is of right or left ventricular origin, we are justified in assuming that in this instance the stimulation was of the left vagus nerve.

In the studies of Jürgens (10) on the action of the vagus in pigeons, no attention was paid to the differences in the effect of faradic stimulation on the two sides. In his curves, it is always stated which vagus had been stimulated, and from these curves it appears that right vagus stimulation with currents of moderate strength affected the auricles and, occasionally, the ventricles. Sometimes, however, the latter escaped from the increased inhibitory action. With weak currents only negative inotropic ventricular effects were obtained. When the left vagus was stimulated,[10] unequal negative or positive inotropic effects on the auricles, or on both auricles and ventricles, with negative chronotropic effects on both, were obtained.

Recently, Garrey (11) has studied the action of the two nerves on the turtle's heart. He makes it clear that he has not obtained absolute uniformity in his results. After making various tests, he proved that the innervation of the right half of the heart falls to the right vagus nerve, while that of the left half falls to the left. But aside from this anatomical difference, there were qualitative differences found, the most important of which is that the left vagus exercises no chronotropic effect on the whole heart in the usual sense. This is due to the fact that the pace-making mechanism in the turtle's heart is on the right side. Even when stimulation of the left vagus can bring the heart to a standstill, the right caval vein, the pace-maker, still continues to beat. Garrey says that when the heart stops under these circumstances, it is because the left vagus merely depresses conductivity and contractility. He has shown, therefore, qualitative and quantitative changes in the vagus nerves of the turtle's heart, depending on the differences of anatomical distribution of the two nerves.

Another method of approaching the vagus control of the cavities of the heart is a relatively recent one, and few observations on the subject are at hand. It consists in determining the action of the extrinsic cardiac nerves with and without the normal functioning of the sino-auricular node. Flack (12), working in Kronecker's laboratory on the functions of the sino-auricular node, attempted to demonstrate the power of the node over the rest of the heart by showing its relation to the cardiac nerves. He examined both right and left nerves. The experiments bearing on this point were performed on dogs and rabbits. The sino-auricular node was rendered inactive and the vagus or accelerator nerves were then stimulated. To render the node inactive, he poisoned it with atropin and muscarin, froze it with ethyl chlorid, or clamped it with Spencer Wells clamps. In the muscarin tests, the two nerves were not adequately treated, and we shall therefore not concern ourselves with them. In the other experiments, the exclusion of the node from activity was demonstrated, since stimulation of

[10] Jürgens, *loc. cit.*, curves 7 and 12.

the right vagus or the right accelerator in such cases always failed to bring about the usual physiological results. Neither auricles nor ventricles showed any response. Stimulation of the left vagus tended to result, as is usual under normal conditions. The results, which were not as uniform as those obtained on stimulation of the right vagus, are given in table I.

TABLE I.

Treatment of node.	Animal.	Stimulation of vagi.				Stimulation of accelerator.			
		Right.		Left.		Right.		Left.	
		Auricle.	Ventricle.	Auricle.	Ventricle.	Auricle.	Ventricle.	Auricle.	Ventricle.
Atropin	Rabbit (1)	−*	+†	−	+				
		−	−	−	−				
	(2)	o‡	o	−	−				
	Dog (3)	−	−	−	−				
Muscarin	Rabbit (4)	+	+	o	o				
		−	−	o	o				
	Dog (5)	+	+	o	o				
Freezing	Dog (6)	−	−	+	+§	o	o	−	−
	(5)	−	−	o	o	−	−	−	−
	(7)					−	−	+	+
Clamping	Rabbit (8)	−	−	o	o				
	(9)	−	−	−	−				
	(10)	−	−	−	+				
	(11)	−	−	−	−				
	(12)	−	−	−	+				
				+	+				
	Dog (3)	−	−						
	|| (7)	+	+	+	+	−	−	+	+

* — means that the usual physiological effect is not obtained.
† + means that the usual physiological effect is obtained.
‡ o means that this nerve did not act well.
§ means that the left vagus did not act well.
|| means that it is doubtful whether all the node was clamped.

It is evident from Flack's table that (1) the right vagus in no instance produced its normal physiological effect on either the auricles or the ventricles, except in the experiments in which muscarin, which stimulates the vagus, was employed, and in one dog in which the entire node was not clamped off; (2) the action of the left vagus varied slightly; it usualy failed to affect the auricles, and where it did act, it acted on the ventricles rather than on the auricles; (3) the right accelerators never acted at all, and the left accelerator sometimes acted and sometimes did not. In other words, the right nerves in the absence of the node did not act on the auricles, while the left ones did; likewise, the right nerves did not act on the ventricles, although the left nerves did. From the point of view of their action on the node, therefore, it appears that the vagi of the two sides act somewhat differently. When the node was made ineffectual, action of

the right nerve on the heart, barring accidents, was not obtained, while the left nerve still acted, although in an irregular manner.

Finally, the observations of Robinson and Draper (13) show differences in the vagi, when tested in man, analogous to those determined by experimental methods.

DISCUSSION.

In the present paper, an effort has been made to offer a possible anatomical and physiological explanation for the occurrence of auricular tachycardia. To do this it has been necessary to show that the two vagi have somewhat different distributions and functions. That such differences exist we think proven from our own experiments and from those of other writers. These differences are not constant, but, in general, the right vagus has an auricular, while the left vagus has an atrio-ventricular or ventricular distribution. The field of action of the right vagus, however, is not absolutely confined to the auricle, but from the evidence at hand we must assume that the right vagus may affect the ventricle directly by fibers to the ventricle independently of those to the auricles. These fibers may have mainly an inotropic function, but there may be chronotropic fibers as well.

The further important point has been established that the auricular rate is usually incapable of being influenced by vagus stimulation when the sino-auricular node has been removed. In other words, chronotropic vagus action on the auricles is usually, at least, only through this node. On the other hand, in the only instances known in which the right vagus did not control the auricular rate, the sinus node was excluded from activity.

Assuming that the evidence is sufficient to establish the above facts, we have the following possible anatomical explanation of the phenomenon of auricular tachycardia. It might result from one of the following lesions: (1) either the vagus (or certain of the right vagus fibers) is the site of a pathological process, so that the functionally active fibers do not reach the node; (2) or the node itself is pathological and incapable of receiving the vagus fibers or of making effective the stimuli received from the nerve; and, in either case, an ectopic area of stimulus production has assumed the pace-making function. Absence of the sino-auricular node alone will not explain the phenomenon, however, for although

the absence of vagus control is known to raise heart rate, absence of the node is known to lower it. It is generally accepted, however, that, under pathological conditions, other parts of the auricle may acquire a rate even faster than the pace-maker. That the electrical complex in such cases is not changed does not exclude the possibility of an abnormal site of stimulus production, for the new site may be so near to the old one that this change does not occur.

The fact that in Rihl's cases slowing of the ventricles occurred, following pressure on the right vagus, does not speak against this explanation, since, as previously stated, there is evidence that ordinarily some fibers from the right vagus reach the ventricles directly. Further study of similar cases, especially the study of the effect of right and left vagus pressure, will be necessary to show how well the explanation given fits all such cases. Our main purpose now is to draw attention to the differences in function of the two vagi, to indicate the relation of the sino-auricular node to vagus action, and finally to offer a possible explanation of auricular tachycardia.

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EXPLANATION OF PLATE 6.

In these curves, the upper line represents the time marked in half seconds; the second line represents auricular systole; the third line, ventricular systole; the fourth line is a signal showing vagus stimulation.

Experiment 581.—March 7, 1911. In this experiment, the sino-auricular node has been excised, and the ventricle made to fibrillate by faradization. The auricular rhythm remained regular.

FIG. 1. Stimulation of the left vagus was without effect. The significance of the removal of the node and the faradization of the ventricle will be discussed in another place.

FIG. 2. Stimulation of the right vagus nerve produced a marked negative inotropic, but no chronotropic effect.

Experiment 584.—March 16, 1911. The curves were taken with the heart intact.

FIG. 3. The right and left vagi were stimulated at 0. Stimulation of the right vagus had a negative chronotropic effect on the whole heart and a negative inotropic effect on the auricle. Stimulation of the left vagus was ineffectual.

Experiment 585.—March 21, 1911. The curves were taken with the heart intact.

FIG. 4. Right vagus stimulation at 5. There is complete stoppage of auricles and ventricles.

FIG. 5. Left vagus stimulation at 5 produced a negative chronotropic effect on the auricles, but a complete cessation of the ventricles.

FIG. 6. The same as figure 4.

FIG. 7. The same as figure 5. Three auricular contractions are followed by ventricular beats. The *A-V* intervals are about the same during as before stimulation.

Experiment 586.—March 24, 1911.

FIG. 8. Left vagus stimulation at 5 is ineffectual.

FIG. 9. Right vagus stimulation at 5 stops the heart.

FIG. 10. Left vagus stimulation at 2 has slight negative chronotropic and marked negative inotropic effect. The ventricle is asystolic; the irregularities of the curve were communicated by the lever of the signal. The curves in this experiment were taken with the heart intact.

Experiment 587.—March 27, 1911. The curve was taken with the heart intact. Ventricular fibrillation has been induced by faradization. There was complete irregularity of the auricles.

FIG. 11. Left vagus stimulation at 5 caused marked negative inotropic effect on the auricles.

FIG. 12. Right vagus stimulation at 5 produced no effect. The nerve had been severely handled in dissection.

FIG. 13. Right vagus stimulation in the thorax at 5. There was complete arrest of the auricles.

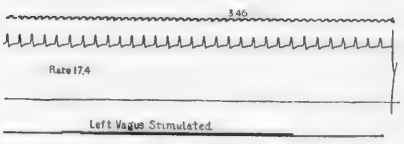


FIG. 1.

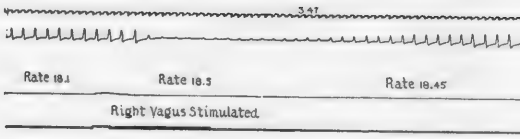


FIG. 2.

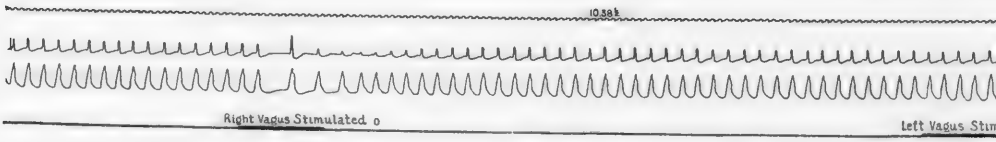


FIG. 3.

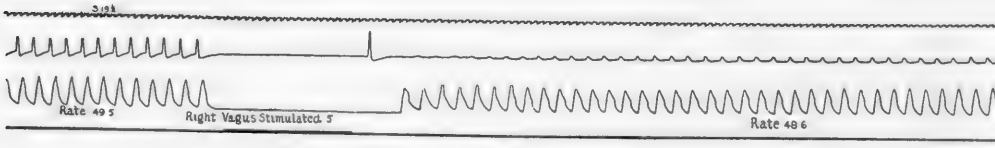


FIG. 4.

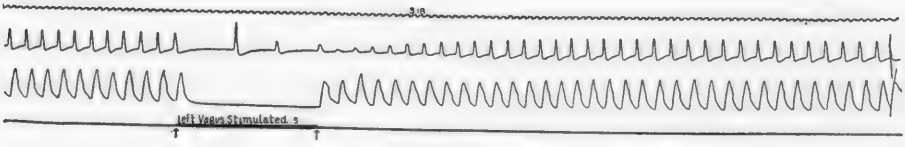


FIG. 5.

FIG. 6.

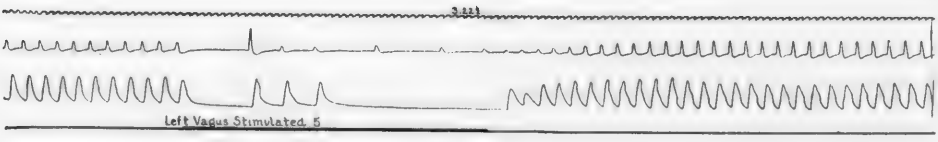
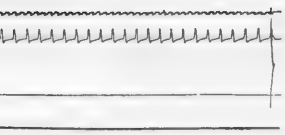


FIG. 7.



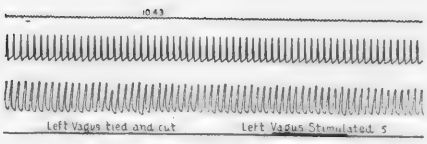
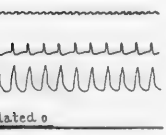


FIG. 8.



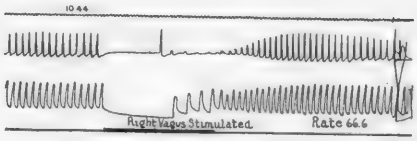
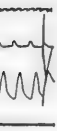


FIG. 9.



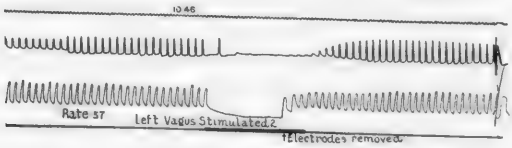


FIG. 10.

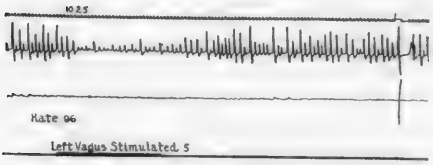
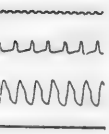


FIG. 11.



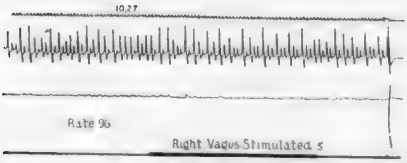


FIG. 12.

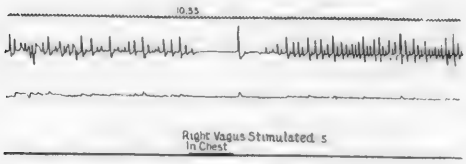


FIG. 13.

THE EFFECTS OF SPLENECTOMY UPON THE CELL CONTENT OF THE THORACIC DUCT LYMPH IN THE DOG, AND ITS RELATION TO THE ACTION OF PILOCARPIN.*

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In recent papers from this laboratory, references to which are given below, various observations on the cytology of the thoracic duct lymph under normal and under definite induced conditions have been made. To these reports are appended complete bibliographies of the subjects.

In anticipation of occasional reference to these papers in their relation to the work here reported, it seems desirable to summarize briefly in the words of the authors some of the points established.

"Muscular activity (struggle) produces a prompt increase in the output of lymphocytes through the thoracic duct" (Rous (1)).

"The intravenous injection of pilocarpine nitrate causes in the dog a rapid and considerable increase in the output of lymphocytes through the thoracic duct" (Rous (2)).

Intramuscular injection of pilocarpin hydrochlorid is followed immediately by an increase in the number of cells in the thoracic duct lymph of the dog. "This increase is interrupted by the action of Roentgen irradiation, and the number can be reduced to below the initial count" (Dixon (3)).

In determining variations from relatively normal cell counts of the lymph, Rous's (4) data given in his paper, "Some Differential Counts of the Cells in the Lymph of the Dog," have been used.

In the course of these investigations, the relations which the spleen might bear to the processes involved and to the end results were considered to be of more than a passing interest. This was especially true in estimating the effects of pilocarpin on the lymph

* Received for publication, November 2, 1911.

stream. The probable methods and seats of action by which lymphocytosis is induced by pilocarpin, have been cited and discussed in the papers referred to.

Previous to the reports cited above, the effects upon the blood of dogs of splenectomy and thoracic duct fistula, each alone and in combination, were observed and reported by Biedl and Decastello (5), Selinoff (6), Crescenzi (7), and Parodi (8), but the effects of splenectomy upon the cell content of the thoracic duct lymph have not been reported heretofore. Neither do I find discussed the results of splenectomy upon the effects of pilocarpin injections nor the effects of pilocarpin upon the results of splenectomy. These three points have been studied in the experiments to be described.

The accompanying tables are intended to be, to a great degree, self-explanatory, thus making unnecessary any detailed description of the results or of the steps in the various processes.

In all, ten experiments were made on ten animals. In each experiment, it was possible to obtain observations upon at least two of the points in question. The ten experiments will be taken up in the order in which they were carried out, and will be accompanied by such interpretations of the methods and results as seem warranted.

Dogs were used in all experiments. In every instance in which the animals were allowed to recover after the operation, as in cases of splenectomy to be followed later by an examination of the lymph, ether or chloroform was administered, and the operations were performed under regular surgical conditions. In the other cases, chloretone served as the anesthetic, and was administered in two gram doses either in alcoholic solution by means of a stomach tube, or, as was found to be equally effective and more convenient, in crystal form in one gram capsules. In all operations, care was taken to prevent any unnecessary hemorrhage. The exposure of the thoracic duct, and the insertion of the cannulas were done after the methods I have described in a former paper. Lymph smears and counts were made from drops as they appeared at the end of the cannula. These were stained by Wright's method, and by methylene-blue after fixation in absolute alcohol and ether. In only two instances was there any clotting within the cannula, and

in both of these there had been a departure from our custom of keeping the cannulas in sulphuric acid and then washing them in distilled water.

The first three experiments were planned to demonstrate the late effects of splenectomy upon the lymph, also the effects of pilocarpin upon the lymph cell count a number of months after splenectomy. These three experiments are so much alike that they may be considered together.

At periods of eight, eight and one-half, and nine and one-half months, respectively, after splenectomy, thoracic duct fistulas were established and the routine examinations of the lymph and blood were made.

By reference to the tables, it is seen that twenty to thirty minutes after the insertion of the cannulas the total cell counts of the lymph cells in these three dogs were 7,850, 5,500, and 9,000 cells per cubic millimeter of lymph. In view of the fact that no counts

TABLE I.

Thoracic Duct Cannula Inserted Eight Months after Splenectomy.

Time.	Lymph.					Blood.				Pilocarpin hydrochlorid.
	Total cells per c.mm.	Small mononuclears.	Large mononuclears.	Polymorphonuclears.	Total of cells counted.	Total white cells per c.mm.	Mononuclears.	Polymorphonuclears.	Total of cells counted.	
20 minutes after insertion of thoracic duct cannula	7,850	427	46	8	481	17,000	110	420	530	
20 minutes after insertion of thoracic duct cannula										15 mg.
35 minutes after insertion of thoracic duct cannula	9,500	484	47	8	539	18,750	100	410	510	
35 minutes after insertion of thoracic duct cannula										30 mg.
55 minutes after insertion of thoracic duct cannula	9,900	430	29	9	468	22,500	91	370	461	

TABLE II.

Thoracic Duct Cannula Inserted Eight and One-half Months after Splenectomy.

Time.	Lymph.					Blood.				Pilocarpin hydrochlorid.
	Total cells per c.mm.	Small mononuclears.	Large mononuclears.	Polymorphonuclears.	Total of cells counted.	Total white cells per c.mm.	Mononuclears.	Polymorphonuclears.	Total of cells counted.	
30 minutes after insertion of thoracic duct cannula	5,500	430	20	4	454	9,750	140	370	510	
30 minutes after insertion of thoracic duct cannula										15 mg.
45 minutes after insertion of thoracic duct cannula	6,200	420	22	8	450	10,750	130	382	512	
45 minutes after insertion of thoracic duct cannula										30 mg.
65 minutes after insertion of thoracic duct cannula	7,800	375	17	4	396	14,900	139	391	530	

of cells had been made before or at the time of the splenectomy, any change effected must be ascertained by comparison with counts made upon other animals.

By comparison with Rous's table (4) it appears that these counts, both total and differential, come well within normal limits. From this we are led to believe that upon the cell count splenectomy has no effect which is persistent through or after a period of from eight to nine and one-half months.

For the blood counts practically the same can be said. When we consider the wide range of variation in the total white cell count which might be brought about during the course of nine months by other factors than splenectomy, the differences here noted have little value. If counts had been made frequently during the interval, some significant change might have been discovered. Among the observers, however, on this point, there is a wide diversity of opinion.

TABLE III.

Thoracic Duct Cannula Inserted Nine and One-half Months after Splenectomy.

Time.	Lymph.					Blood.				Pilocarpin hydrochlorid.
	Total cells per c.mm.	Small mononuclears.	Large mononuclears.	Polymorphonuclears.	Total of cells counted.	Total white cells per c.mm.	Mononuclears.	Polymorphonuclears.	Total of cells counted.	
30 minutes after insertion of thoracic duct cannula	9,000	270	16	5	291	12,000	110	238	348	
30 minutes after insertion of thoracic duct cannula										15 mg.
45 minutes after insertion of thoracic duct cannula	11,200	310	20	4	334	11,500	93	291	384	
45 minutes after insertion of thoracic duct cannula										30 mg.
65 minutes after insertion of thoracic duct cannula	12,700	460	34	7	501	14,800	121	406	527	

After material had been collected for these preliminary counts, an intramuscular injection of fifteen milligrams of pilocarpin hydrochlorid was administered. Fifteen minutes later, counts of cells showed an increase in the lymph and in the blood, except in the case of table III where there was a slight decrease in the total white blood cells. The increase in cells in the lymph is relatively greater than in the blood. Several counts were made during the next twenty minutes but showed no change beyond the usual variation in successive cell counts.

Fifteen minutes after the first administration of pilocarpin, a second quantity, this time thirty milligrams, was given in the same manner. Counts following this administration showed a further increase in the number of cells. The total increase secured by both doses of the drug varied between 1,000 and 2,000 for the lymph, and 2,000 and 5,200 for the blood. These were relatively

large doses of pilocarpin. The increase secured is, at first consideration, somewhat marked, but upon comparison with the changes that pilocarpin induces in dogs that have not been previously treated as these animals had, it is relatively slight, for, in the control experiments cited, it was not uncommon to obtain an increase of more than 100 per cent.

We are led to think, then, that by removing the spleen, we have interfered with some important factor in the effects of pilocarpin upon the cells of the lymph and the blood. In addition to this, we may as reasonably think that some factor in the action of the drug remains which tends to increase the mononuclear cells of the lymph and both the mononuclear and the polymorphonuclear elements in the blood. A consideration of these factors will be taken up later.

At autopsy, these three dogs revealed nothing of note. The hemolymph-nodes could not be said to be increased in number or size. The effects in this respect are not at all comparable to the results of splenectomy in sheep, as reported by Professor Warthin (9).

In general, then, from these three cases we may say that: (1) splenectomy has no appreciable effect upon the cell content of the thoracic duct lymph after a period of eight or nine months; (2) the effect of pilocarpin upon dogs splenectomized eight or nine months previously, is a definite increase in the number of the cells in the thoracic duct lymph, but this increase is not so marked as in dogs which have not been splenectomized, as has been shown by former sets of investigations done in this laboratory.

The next three experiments were intended to exhibit the earlier effects of splenectomy upon the cell content of the duct and the action of pilocarpin. Accordingly, three dogs were splenectomized and then subjected to routine examinations for one, two, and four weeks, respectively. The conditions developing are shown in tables IV, V, and VI.

The comparison of the blood cell counts made at the time of the first and second operations, shows no uniformity in change. It will be seen by reference to tables IV, V, and VI, that two dogs underwent a decrease in white blood cells, and one showed an increase. The cases showing a decrease were examined one and two weeks, respectively, after splenectomy, while the case showing an

increase was examined four weeks after the preliminary operation. From this we should not presume to conclude that the immediate

TABLE IV.

Thoracic Duct Cannula Inserted One Week after Splenectomy.

Time.	Lymph.					Blood.				Pilocarpin hydrochlorid.
	Total cells per c.mm.	Small mononuclears.	Large mononuclears.	Polymorphonuclears.	Total of cells counted.	Total white cells per c.mm.	Mononuclears.	Polymorphonuclears.	Total of cells counted.	
At time of splenectomy						11,400	120	417	537	
30 minutes after insertion of thoracic duct cannula	4,300	364	30	2	396	9,500	74	221	295	
30 minutes after insertion of thoracic duct cannula										20 mg.
60 minutes after insertion of thoracic duct cannula	6,800	471	42	6	519	14,900	114	362	476	

TABLE V.

Thoracic Duct Cannula Inserted Two Weeks after Splenectomy.

Time.	Lymph.					Blood.				Pilocarpin hydrochlorid.
	Total cells per c.mm.	Small mononuclears.	Large mononuclears.	Polymorphonuclears	Total of cells counted.	Total white cells per c.mm.	Mononuclears.	Polymorphonuclears.	Total of cells counted.	
At time of splenectomy						20,250	110	420	530	
30 minutes after insertion of thoracic duct cannula	6,000	359	26	381	386	9,400	101	320	421	
30 minutes after insertion of thoracic duct cannula										20 mg.
60 minutes after insertion of thoracic duct cannula	7,900	410	39	1	450	10,000	143	422	565	

TABLE VI.

Thoracic Duct Cannula Inserted Four Weeks after Splenectomy.

Time.	Lymph.					Blood.				Pilocarpin hydrochlorid.
	Total cells per c.mm.	Small mononuclears.	Large mononuclears.	Polymorphonuclears.	Total of cells counted.	Total white cells per c.mm.	Mononuclears.	Polymorphonuclears.	Total of cells counted.	
At time of splenectomy						13,500	66	217	283	
30 minutes after insertion of thoracic duct cannula	2,800	436	29	1	466	16,000	112	421	533	
30 minutes after insertion of thoracic duct cannula										20 mg.
60 minutes after insertion of thoracic duct cannula	4,400	388	36	4	428	18,000	96	430	526	

effect of splenectomy is a reduction of the white cell count to be followed by a secondary rise after three or four weeks. Yet this is suggested when we take into consideration the fact that an increase in white cells in the blood is the usual sequence of an abdominal operation upon a dog. These decreases in cells would seem to be attributable to the removal of the spleen, and to be greater than the figures show on account of having to offset the usual rise in the cell count. To form any definite basis for such conclusions, it would have been necessary to make counts at frequent intervals, and to take into consideration the healing of the laparotomy wounds, the possibility of infection, the food furnished the animals, and all factors likely to effect changes in the cell counts.

On this point, observers have reached no uniform conclusion, for either their results have lacked uniformity or secondary factors were not taken into consideration in all cases.

On turning our attention to the lymph, we note by comparison with our adopted standards of normal counts, that the numbers fall within normal limits, but are relatively low.

When we compare tables IV, V, and VI, with tables I, II, and

III, we find a definite relative decrease in the cells in the former, *i. e.*, in the animals splenectomized for only one to four weeks. In these, the average cell count is 3,000 or more per cubic millimeter less than in tables I, II, and III. It seems apparent, then, that the removal of the spleen establishes a lower cell count, but that this does not persist more than a few months.

In each of these cases, twenty milligrams of pilocarpin hydrochlorid were injected into the thigh muscles. Counts made thirty minutes later showed a definite rise in the number of both blood and lymph cells. These increases are practically the same in amount as in tables I, II, and III. But by comparison with the tables of dogs that had not been splenectomized, one must conclude that the effect is not marked. So it appears that soon after splenectomy—one to four weeks—the effect of pilocarpin is also much less than in non-splenectomized dogs.

In the next experiment, table VII, a cannula was inserted into the thoracic duct of a normal dog.

TABLE VII.

Insertion of Thoracic Duct Cannula Followed by Splenectomy.

Events and time.	Lymph.					Pilocarpin hydrochlorid.
	Total cells per c.mm.	Small mononuclears.	Large mononuclears.	Polymorphonuclears.	Total of cells counted.	
30 minutes after insertion of thoracic duct cannula	2,500	320	30	7	357	
30 minutes after insertion of thoracic duct cannula						20 mg.
60 minutes after insertion of thoracic duct cannula	4,800	447	51	5	503	
60–65 minutes after insertion of thoracic duct cannula, spleen removed						
10 minutes after removal of spleen	5,250	241	10	1	252	
30 minutes after removal of spleen	3,000	310	16	4	330	

Thirty minutes after the insertion of the cannula into the thoracic duct, the cell count of the lymph was 2,500 per cubic millimeter. Twenty milligrams of pilocarpin hydrochlorid were then injected deeply into the thigh muscles. At the end of half an hour, the count had nearly doubled, reaching 4,800 per cubic millimeter. Then the

spleen was quickly removed without loss of blood and with as little manipulation of the tissues as possible. This required about five minutes. The cell count was 5,250 per cubic millimeter, or more than 100 per cent. increase over the original count. Twenty minutes later the number had fallen to 3,000 per cubic millimeter, or to nearly the original number.

This experiment was planned to show the effect of splenectomy upon the changes induced by pilocarpin. From the figures, it appears that the pilocarpin brought about the usual increase of cells, which continued after the secondary operation, or was augmented thereby. Then, sooner than usual after this amount of the drug, the number of cells fell rapidly. It might be considered, therefore, that splenectomy inhibited in some degree the effects of the drug. This point will be discussed later in connection with other experiments made under equivalent conditions.

The first part of the next experiment, table VIII, is a duplicate of the preceding one.

TABLE VIII.

Insertion of Thoracic Duct Cannula Followed by Splenectomy.

Events and time.	Lymph.					Pilocarpin hydrochlorid.
	Total cells per c.mm.	Small mononuclears.	Large mononuclears.	Polymorphonuclears.	Total of cells counted.	
30 minutes after insertion of thoracic duct cannula	4,000	218	31	3	252	
30 minutes after insertion of thoracic duct cannula						20 mg.
50 minutes after insertion of thoracic duct cannula	6,500	335	42	7	384	
50–55 minutes after insertion of thoracic duct cannula, spleen removed						
15 minutes after removal of spleen	7,100	360	35	4	399	
35 minutes after removal of spleen	3,400	240	22	4	266	
35 minutes after removal of spleen						20 mg.
55 minutes after removal of spleen	3,500	312	30	6	348	

The preliminary count of 4,000 per cubic millimeter increased in twenty minutes after administration of twenty milligrams of pilocarpin to 6,500. Then the spleen was removed, and a count taken fifteen minutes later showed 7,100 cells per cubic millimeter.

Twenty minutes after this count, the number had fallen below the original count.

This again argues that by removing the spleen, we have interfered with the action of the pilocarpin, because, from former experience, we should not expect the effect of the drug to be exhausted so soon. However, it occurs that the point can be more definitely ascertained by administering a second dose of pilocarpin. Accordingly, a second dose of twenty milligrams was injected and the effects noted. After a period of twenty minutes, there was practically no change in the cell count, there being an increase of only one hundred cells per cubic millimeter.

This adds weight to the conclusion that the spleen is an important factor in bringing about the lymphocytosis induced by pilocarpin. The usual experience has been that a second administration approaches nearly the first count. In this instance, it is fair to say that the action of the drug was apparently inhibited by the absence of the spleen.

In the preceding experiments, we have shown by numbers the effects of splenectomy, both late and relatively early, upon the cell content of the thoracic duct. We have also demonstrated the effect of pilocarpin after splenectomy, and, in the last experiment, the effect of pilocarpin before and after splenectomy.

TABLE IX.

Insertion of Thoracic Duct Cannula Followed by Splenectomy.

Events and time.	Lymph.					Pilocarpin hydrochlorid.
	Total cells per c.mm.	Small mononuclears.	Large mononuclears.	Polymorphonuclears.	Total of cells counted.	
30 minutes after insertion of thoracic duct cannula	8,600	291	41	0	332	
30–35 minutes after insertion of thoracic duct cannula, spleen removed						
10 minutes after removal of spleen	9,750					
20 minutes after removal of spleen	8,000					
30 minutes after removal of spleen	6,200					
30 minutes after removal of spleen						20 mg.
50 minutes after removal of spleen	7,800					
70 minutes after removal of spleen	5,000	310	43	4	357	

The next step was to ascertain the immediate effect of splenectomy and how this effect is modified by pilocarpin.

The usual count was made of the cells in the thoracic duct lymph of a dog that had not been treated in any way. This count shows 8,600 per cubic millimeter. The spleen was then removed and the next count gave 9,750 cells per cubic millimeter, or an increase of over 1,000 cells per cubic millimeter. The next count, made ten minutes later, showed a decrease to 8,000 per cubic millimeter, and still later to 6,200 per cubic millimeter.

This then may be said to show that the early effect of splenectomy is an increase in cells in the lymph, followed very soon by a decrease which may carry the curve below the original count.

After making these observations, twenty milligrams of pilocarpin hydrochlorid were given, and at the end of twenty minutes the number had again gone up to 7,800 per cubic millimeter. This number was not maintained for any length of time, but in the next twenty minutes it had decreased to 5,000 per cubic millimeter. This action of pilocarpin is in harmony with the findings in similar circumstances in the preceding experiments.

In every case in which cell counts preceded and followed the operation of splenectomy, it appears that there is a definite rise in the number of cells immediately after the operation. This is true both with and without the association of pilocarpin. Although the operations were done with as little manipulation of the organs as possible, it was conceived that the mechanical factor involved in the handling of the spleen might to some degree be accountable for this effect.

It was determined therefore to massage the spleen in order to see if similar results would be obtained. After ascertaining the cell count of the lymph, the spleen was exposed and subjected to squeezing and manipulations. This was kept up for five minutes, after which the organ was dropped back into place.

The next count, ten minutes later, was as large as if a full dose of pilocarpin had been administered, for it showed 6,100 cells per cubic millimeter, whereas the original count was 3,800 cells per cubic millimeter. This is undoubtedly evidence that contractions of the spleen may be a great factor in increasing and in maintaining the cell content of the lymph.

TABLE X.

Insertion of Thoracic Duct Cannula; Splenic Massage; Splenectomy.

	Lymph.	Pilocarpin hydrochlorid.
	Total cells per c.mm.	
30 minutes after insertion of thoracic duct cannula	3,800	
30–35 minutes after insertion of thoracic duct cannula, spleen exposed and massaged		
45 minutes after insertion of thoracic duct cannula	6,100	
55 minutes after insertion of thoracic duct cannula	5,000	
55 minutes after insertion of thoracic duct cannula		20 mg.
65 minutes after insertion of thoracic duct cannula	6,400	
75 minutes after insertion of thoracic duct cannula	7,000	
75–80 minutes after insertion of thoracic duct cannula, spleen removed		
10 minutes after removal of spleen	5,500	
10 minutes after removal of spleen		20 mg.
25 minutes after removal of spleen	5,000	

Ten minutes later the number of cells had fallen to 5,000 per cubic millimeter, and twenty milligrams of pilocarpin were given. The number of cells increased to 7,000 per cubic millimeter. The spleen was then removed, and the next count, ten minutes later, showed 5,500 per cubic millimeter. Another dose of pilocarpin was given, but it failed to increase the number of cells.

The general consideration of these experiments falls naturally under three heads: (1) the effects of splenectomy, immediate, early, and late, upon the thoracic duct cell content; (2) the effects of pilocarpin hydrochlorid upon the conditions existing after splenectomy; (3) the effects of splenectomy upon the conditions induced by pilocarpin hydrochlorid.

In addition to these three topics, we shall consider the factors involved in the action of pilocarpin.

(1) The lymphocytes in the blood after splenectomy have been counted and studied. Delamere (10) refers to the results of various workers and to the differences in their observations. He mentions the work of Uskow (11), Emilianoff (12), Hartmann (13), and Vaquez (14), who found the lymphocytes increased after splenectomy, and to Koroboff (15), who found them diminished. If we are to adopt the statement that most or perhaps all of the cells of this type in the blood are furnished it by way of the thoracic duct lymph, then we may assume that alterations in the lymph content

will be manifested by changes in the blood content, and, on the other hand, that alterations in the blood content are consequences of alteration in the lymph content. These interdependent relations are found not to be constant, and should not be assumed to be so when we consider that lymph flow, cell destruction in the blood, and the ratio of the supply of these cells to their destruction in the blood, are not constant.

Yet it would appear reasonable to think that, accompanying or immediately preceding an increase in the lymphocytes in the blood, there is a relatively equal increase of these cells in the thoracic duct lymph.

By an examination of the tables, it appears that in all cases in which counts were made immediately before and after splenectomy, there is developed an increase in the number of these cells. This, however, is not permanent, and does not add to the sum total of the effect of pilocarpin. This is shown in tables VII, VIII, and IX. In tables VII and VIII, splenectomy was preceded by the administration of pilocarpin, and the increase might be construed as the further effect of the drug. But in table IX, splenectomy was not preceded by pilocarpin, and yet the increase is evident. This increase of short duration following each operation, gave rise to the thought that it might be due entirely to a mechanical factor; namely, the manipulation of the spleen and intestines. In experiment 10, the mechanical element was made prominent, and the results were very suggestive. An increase similar to that induced by pilocarpin occurred. If allowance is made for the time necessary for the duct to empty, we see that this increase was not persistent after the manipulation.

From these experiments I should conclude that the increase in lymphocytes in the blood and lymph immediately after an operation involving the handling of the spleen and intestines, is of purely mechanical origin. In conjunction with this, I may suggest that the action of pilocarpin in increasing the lymphocytes is mechanical, inducing contractions of the spleen and increasing the peristaltic movements of the intestines.

Furthermore, in experiment 10, it appears that the final removal of the spleen caused no increase in cells. This is not opposed to

the view just stated, because in this case, the spleen had been subjected to vigorous massage and then to the action of twenty milligrams of pilocarpin. In that condition, it may be said to have been emptied of the cells which would have been expressed into the lymph circulation by the operation.

The early effect of splenectomy is ascertained only by a comparison with former general observations. With this in mind, we are led to believe that after intervals of one to four weeks, there is a relatively low cell content. This is probably to be explained on the basis of the absence of a white cell-forming organ. The first parts of tables IV, V, and VI give the data upon which this is based.

The late effect of splenectomy is to be seen in experiments 1, 2, and 3. As these counts fall well within normal limits, we must conclude that the operations had no lasting effects. To explain this, we may assume that there was a compensatory action on the part of other white cell-forming organs. The compensatory action of the hemolymph-nodes after splenectomy has been established by Dr. Warthin (9). In my cases, there was no such increase in the size and number of hemolymph-nodes as he secured in sheep. However, in two of the dogs, tables I and III, there were more and larger hemolymph-nodes than are usually found in these animals. In case 2, close search did not reveal any such nodes. It is perhaps significant that the lymph cell count in this dog was less than in the other two.

Another circumstance in this animal was very marked. Although dog 2 was operated upon at the same time with the others, and although its wounds healed perfectly, and it appeared to do equally well at first, it soon developed a marked degree of emaciation, and we found it imposible to improve his condition. Dogs 1 and 3, on the other hand, became quite fat and remained so during the time they were kept. Dog 2 was kept well supplied with food and water, and it was often noted that he devoured more food than the other dogs. He became vicious and killed several of the other dogs in his pen. Whether or not his inability to thrive on the food supplied to him was in any way related to the failure of the hemolymph-nodes to develop is not known. It is simply mentioned in this connection

because the condition was strikingly different from that found in the other cases.

(2) The effects of pilocarpin hydrochlorid after splenectomy were demonstrated in all ten cases. With the single exception of the last, there developed an increase in the number of cells in the lymph. Tables I to IX show an immediate response to the action of pilocarpin. The striking feature of this increased number of cells is that the rise in the count did not nearly attain the height usually obtained by injections of pilocarpin in dogs not previously treated.

This was equally true in those cases in which, several months after splenectomy, the cell count had returned to normal. It is evident, then, that in estimating the importance of the spleen as a factor in the action of pilocarpin, the effects seen after splenectomy must be deducted from the total increase of cells in non-splenectomized animals.

We believe that the whole effect of pilocarpin in increasing the number of cells can be explained on a purely mechanical basis, as has been pointed out in the reports already cited from this laboratory. After one site of this mechanical action, together with an important source of the cells under consideration, is removed by splenectomy, we still have left regions in which this force is operative. Increased respiratory activity is an important factor that has been emphasized before. But there is another factor to which we find that attention has not been called, *i. e.*, the marked increase in peristaltic action of the intestines.

In the walls of the intestines and in the mesentery is situated a relatively broad channel of lymph-vessels. Under the increased activity of the musculature of the intestine, the flow of lymph is increased. At the same time, there is a great escape of fluid into the lumen of the intestine. As a consequence, not only is the flow of lymph increased, but the lymph itself, as well as the blood, undergoes condensation due to loss of fluid.

The deduction from the experiments, viewed in this relation, is that, after splenectomy, injection of pilocarpin causes an increase of cells in the thoracic duct lymph, which is to be explained by the increased activity of the respiration and especially of the coils of the intestines.

(3) The effect of splenectomy upon the effects induced by pilocarpin is demonstrated in several cases. As has been pointed out, immediately after the operation there is an increased output of cells. This has been explained as being a consequence of the manipulation of the spleen. Very soon, however, there results a decided drop in the count, which we consider due to the removal of an important site of cell formation and of mechanical activity. Not all this decrease is to be attributed to the removal of the spleen, for we must realize that the other mechanical factors are gradually declining in their efficiency at the same time. It is evident, though, that removal of the spleen after the administration of pilocarpin hydrochlorid lessens materially the effect of the drug as measured by the number of cells in the thoracic duct lymph.

CONCLUSIONS.

1. Splenectomy in dogs results in an immediate increase of cells in the thoracic duct lymph, due to the manipulation involved in the operation. This increase is soon followed by a low cell count, attributable to the removal of an important source of the cells under consideration. Several months after the operation, a normal cell count is reëstablished, due probably to a readjustment of activity on the part of other cell-forming tissues.

2. Pilocarpin injections after splenectomy result in an increase of cells, attributable to an increased activity of the respiratory organs and of the intestine.

3. After the injection of pilocarpin, splenectomy may increase the cell count, but it causes a decrease sooner than in cases of pilocarpin injection not followed by splenectomy.

In the preparation of this work, I am under obligations to Professor Warthin for a general oversight of the problem; to Dr. Morse, of the Pathological Department, for much help in the routine of many of the experiments, and for making cell counts; and to Dr. Kollig and Mr. Hillman for assistance given several times in the splenectomy operations.

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CULTURAL STUDIES ON MOUTH SPIROCHÆTÆ (TREPONEMA MICRODENTIUM AND MACRODENTIUM).*

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Plates 7–11.

In the normal oral cavity of man and animals, three distinct types, at least, of spiral organisms have long been recognized. The first type is much larger and thicker than the other two and has only a few flat curves. It is known as *Spirochæta buccalis* (Cohn, 1875). The second and third types are more delicate in structure and possess a thin flexible body and quite regular, closely set shallow curves, the difference between the two being that one is smaller than the other. The smaller type is called *Spirochæta dentium* (Koch, 1877), while the medium form bears no particular name, but has been designated the "medium form" by Hoffmann and von Prowazek.[1]

The relative number of the three types of spirochætæ varies greatly according to the conditions of the mouth and to the localities from which the material is obtained. Thus, in my experience, the smallest type is more abundant in the deposit at the juncture between the teeth and the gum, and in the cavity of a carious tooth; while the medium and buccalis types are more frequently found in the mucus about the tonsils and pharynx, and in large numbers in ulcerative stomatitis.

As regards the pure cultivation of these organisms, our knowledge has been very meager. The only investigator who has succeeded in isolating them from associated bacteria in a culture is

* Received for publication, November 6, 1911.

[1] Hoffmann, E., and von Prowazek, S., Untersuchungen über die Balanitis und Mundspirochaten, *Centralbl. f. Bakteriol., 1te Abt.*, 1906, xli, 741, 817.

Mühlens (1906).[2] The descriptions of his cultivated spirochætæ agree, in part, with the morphological features of *Spirochæta dentium* (Koch) and, in part, with those of the medium type. From this fact, Mühlens suggested that the medium type is nothing more than forms that, under certain conditions, spring out of the smaller type. However, he left this point unsettled. As the work to be presented in this paper shows, I was able to obtain at least two distinct types of mouth spirochætæ in pure cultures. One of them is the smaller type and has affinities with *Spirochæta dentium* (Koch), and the other belongs to the medium type. The morphological and cultural characteristics of these two organisms are so distinct that no confusion of the two should be made. It is not improbable that the culture of Mühlens contained both the smaller and the medium types. In fact, on two occasions, I have obtained such a mixed culture.

Hitherto, on account of the lack of pure cultures, a reliable means of differentiating various spirochætæ was impossible, and a loose terminology, such as has been in use for the spirochætæ of the mouth, was all that could be employed without risk. But, now that successful separation in pure cultures of morphologically distinct forms has been accomplished, it is possible to define two distinct species of mouth spirochætæ. I therefore propose the name of *Treponema microdentium* for the smaller, and *Treponema macrodentium* for the medium type. My reason for including these two types under the general specific name of "dentium" with the prefixes of "micro" and "macro" is that they belong unquestionably to the same genus, while the buccalis type belongs rather to another genus. In general characteristics, the micro- and macrodentium agree with the genus *Treponema* (Schaudinn) to which also the pallidum belongs.

CULTIVATION OF TREPONEMA MICRODENTIUM.

A small amount of tooth deposit, preferably from a young child, is put in a tube containing a large quantity of sheep serum water

[2]Mühlens, P., Ueber Züchtung von Zahnspirochäten und fusiformen Bacillen auf künstlichen (festen) Nährböden, *Deutsch. med. Wchnschr.*, 1906, xxxii, 797; Mühlens, P., and Hartmann, M., Ueber Bacillus fusiformis und Spirochæte dentium, *Ztschr. f. Hyg. u. Infectionskrankh.*, 1906–07, lv, 81.

and a piece of sterile tissue (testicle or kidney of rabbit or sheep).[3] The culture medium thus inoculated is now covered with a layer of sterile paraffin oil. It is best to inoculate about three tubes which are incubated for about ten days at 37° C. During this time, the medium may become firmly or loosely coagulated through the action of bacteria simultaneously growing in the culture; a small amount of the culture is taken with a capillary pipette from the bottom of the tube and examined under the dark-field microscope. Numerous active small spirochætæ and other bacteria are usually seen. The next step is to purify the spirochætæ from the accompanying bacteria. The same principle is employed as for the purification of the pallida. For this purpose, a series of wide and tall tubes containing serum agar, in the ratio of 1 to 3, and a piece of sterile tissue (preferably sheep or rabbit) are inoculated with a capillary pipette with the impure fluid culture by inserting it carefully into the agar along the middle line until the point reaches the bottom of the tube. Then the contents of the pipette are gently forced in by means of a rubber bulb or a syringe, care being taken not to split the agar column by means of the compressed air. It is best not to empty the pipette within the agar, but to withdraw it with some of the contents still present. After withdrawal, the agar is covered with a layer of paraffin oil and the tubes are put in a thermostat. In ten days or two weeks, the tubes begin to show a hazy colony of spirochætæ spreading out from the stab canal that is now filled up with a whitish mass of bacterial colonies. When the size of the hazy colony becomes large enough to allow fishing, without risk of touching the stab canal, the tube is cut, the agar column split across very gently, and a sterile capillary pipette carefully inserted into the hazy zone from the clean newly cut surface of the medium. The material removed shows, under the dark-field, the presence of innumerable active spirochætæ. Pure cultures can then be obtained by inoculating a new medium with this colony by means of a capillary pipette. It may, of course, happen that purification may not at first be successful, so that it is necessary to repeat the same procedure until success is obtained.

[3] Noguchi, *Jour. Am. Med. Assn.*, 1911, lvii, 102; *Jour. Exper. Med.*, 1911, xiv, 99.

CULTIVATION OF TREPONEMA MACRODENTIUM.

The same technique is used for isolating the microdentium. Special precautions must be taken (1) to obtain material in which the larger dentium organisms are predominating, as from a case of Vincent's angina or ulcerative stomatitis; (2) to enrich the larger type by several transplantations of the impure culture in fluid media; and (3) to select carefully the colonies that are characterized by fainter visibility and slower growth. The pure cultivation of the macrodentium is more tedious than that of the microdentium.

Sometimes it happens that the impure cultures show no, or only a slight coagulation of the serum constituents. In this instance, the purification is comparatively easy because the solid media do not become turbid and the delicate colonies of the spirochætæ radiating from the central stab canal can easily be recognized. But when the bacteria growing in the impure culture, as is the rule, produce acid and perhaps gas, the purification is rendered very difficult because the solid media become so turbid or torn up with gas that the colonies of the spirochætæ are not easily seen, or, when seen are difficult to isolate. Under such circumstances, it is advisable either to select better material for inoculation or to reduce the acid-producing bacteria by leaving the impure cultures for four or five weeks, during which time the spirochætæ remain alive and still transplantable while the bacteria often die. It may, however, be necessary to repeat this procedure several times before successful purification is attained.

Treponema Microdentium.—(Figures 1 to 10.) When cultivated in a serum agar tissue medium, a faint haze becomes perceptible within seventy-two hours at 37° C. along the stab canal. The density and dimensions of the hazy growth increase slowly for many days until the agar column two to three centimeters below the surface becomes diffusely hazy and opalescent. Along the stab canal, the growth becomes so dense that it appears as an irregularly thickened, whitish streak without definite contour. During the first and second weeks, numerous small spots of separate colonies scattered here and there appear around the stab canal, which are often seen to grow in the space between the agar column and the wall of the

test tube. Young cultures have only a faint odor, but after about three weeks, the odor becomes characteristically fetid.

This spirochæta measures less than 0.25 of a micron at the middle of the body and gradually tapers towards both extremities, which are sharply pointed. The length varies according to the age of the culture: young cultures show many short forms, and old ones may reach eight microns in length and show, on the average, fourteen curves. The number of shallow and rectangular curves varies also according to the length of the specimen; but the angle and size of each curve are strikingly constant, although diminishing gradually towards the extremities. The pointed ends are usually drawn out straight along the hypothetical axis. The motion of the spirochætæ is quite active and rotating, and the body is flexible. Under certain conditions, a long thin flagella-like projection is observed at one or both ends. The general appearance of the organism is a straight line. When such a culture is disturbed by a capillary pipette or platinum loop, the regular straight forms become less evident and peculiarly twisted specimens more numerous. In these disturbed specimens, the curves, nevertheless, resemble the original. Singularly enough, these contracted individuals swim swiftly by a serpentine or vibratory motion. When the flagellum of one end is caught by a fixed object, the spirochæta swings actively as if attempting to liberate itself. Longitudinal division has frequently been observed in pure cultures.

As long as oxygen is completely excluded from a fluid medium, straight typical forms are produced; but when the medium is imperfectly anaerobic, the spirochætæ take on irregular and non-typical shapes.

The microdentium causes a loose coagulation of the serum constituents that renders litmus colorless after about two weeks' growth and the cultures exhale a strong fetid odor.

The injection of a culture of this organism into the testicles of rabbits and the subcutaneous tissue over the eye of a *Macacus rhesus* monkey, causes, within twenty-four hours, a marked induration that persists for about a week; however, no spirochætæ were detected in the inflamed tissue.

Treponema Macrodentium.—(Figures 11 to 16.) In the serum

agar tissue medium, after about four or five days at 37° C., it grows as a faint hazy colony which is almost transparent and without demarcation towards the outside. Isolated colonies are rarely produced, and strict anaerobiosis is required for any growth to take place.

According to the condition of the culture, the morphological features of the macrodentium are subject to wider variations than those of the microdentium. In young cultures the organisms are plump and short with rather irregular shallow curves. The extremities taper off abruptly. Under the dark-field, the body of the organism shows double refraction; namely, a feeble line of refraction along the axis, bordered on both sides with stronger zones of refraction. The body is flexible and capable of creeping with great ease through the agar in all directions. When in a clear space, the organism swims freely with swinging or vigorously vibrating movements. Certain specimens have been observed in which the body shows a longitudinal cleft or division, and long delicate flagella-like projections, with minute curves, are attached to one or both ends. Specimens from an older culture are somewhat longer and thinner and taper more gradually towards the ends; the curves are almost rectangular, shallow, and quite regular; the body shows no longer double refraction, except near the middle. Motility seems to be lost in certain individuals, except for a few curves at the extremities that may still exhibit a swinging motion. Young organisms showing double refraction and having two to eight curves may measure 0.7 to 1.0 micron in width and 3 to 8 microns in length. Older specimens having fourteen or more curves measure usually 0.3 of a micron in width and 12 microns or more in length. The spirochætæ grown in a fluid medium show about the same dimensions, but many long specimens, corresponding to the length of several ordinary ones, may appear. Besides these, other individuals occur, the bodies of which are much heavier in the middle and show more closely set, regular, rectangular curves, and of which one fourth to one third of the body toward the extremities gradually tapers off to sharply drawn out points with comparatively few shallow curves.

INVOLUTED FORMS.

I have already stated that when the exclusion of the oxygen is not complete, the microdentium produces many irregularly waved forms in a solid or fluid medium (figures 18 to 23). These individuals swim freely and are actively motile. By transplanting them into a new medium, under strict anaerobic conditions, they reproduce the normal forms. In fact, the forms which are commonly observed in a smear from tooth deposit consist of these irregular individuals; and it is only in an artificial medium, under strictly anaerobic conditions, that the regular straight forms showing a series of uniform curves appear. This fact is also true of the macrodentium.

On one occasion, I observed in an impure culture, in a fluid medium growing under imperfect anaerobic conditions, numerous spiral organisms with extremely regular, almost rectangular curves. Their length varied from four to sixty microns or more, and, with the corresponding number of curves, each averaged 1.8 microns. They were usually straight, finely tapered towards the ends, and apparently showed no real motility. Their bodies were flexible, but no modification of the form of the curves could be made out. The shorter ones, with few curves, are very thin (0.3 of a micron), while the longer specimens may be twice, three times, or four times as thick as the main portions of the body of the smaller varieties. Even with the longest forms, no cross septa, such as are easily recognized in certain other large spirochætæ, were seen. On the other hand, many larger forms showed a central cleft suggesting longitudinal division, which may run along part or the whole of the axis.

Transplantation of such cultures into a new fluid medium, kept under similar conditions of cultivation, causes these peculiar immobile spiral forms to reproduce indefinitely; while when cultivated in a solid medium under strictly anaerobic conditions they fail to grow at all.

Although I am inclined to view this finding as an example of involution of the macrodentium, I have not yet concluded whether we may not have before us rather a new spirochæta than merely an involuted form. The culture in which these spirochætæ appeared came from scrapings of an ulcer of the mouth of a child of three

years of age, in whom ulceration, attended by fever, has been occurring regularly every three weeks since infancy. I am indebted to Dr. La Fetra for access to this case.

CONCLUSIONS.

I have described the successful cultivation of two distinct species of *Treponema* from the oral cavity of man. The species differ from one another morphologically and in respect to their cultural properties. As the two species have now been clearly separated and cultivated for the first time, I propose for them the names *Treponema microdentium* and *macrodentium,* respectively.

Division along the longitudinal axis has been demonstrated for both *Treponema macrodentium* and *microdentium.* When the micro- and macrodentium are cultivated under imperfect anaerobic conditions, they undergo involution, but still continue to vegetate to some extent. Finally, a spiral organism has been cultivated and described that may be merely an involuted form of the macrodentium, or it may be a new species altogether.

EXPLANATION OF PLATES.

PLATE 7.

FIGS. 1, 2, 5, and 6. *Treponema microdentium* in a pure fluid culture, twenty days at 37° C. × 1,400.

FIG. 3. *Treponema microdentium* in a pure solid culture, ten days at 37° C.; a few forms show longitudinal division.

FIG. 4. A preparation of the microdentium from a pure culture in a solid medium, five days at 37° C. × 1,400.

FIG. 5. An exceptionally long chain of the microdentium from a pure culture in a fluid medium.

FIGS. 7 and 8. *Treponema microdentium* from a pure culture in a solid medium, twelve days at 37° C. Giemsa stain. × 1,400.

PLATE 8.

FIG. 9. A schematic reproduction of a dark-field view of the microdentium. A seven day old pure culture in a solid medium. × 1,400.

FIG. 10. The same as figure 8, but the culture has been disturbed by repeated punctures, and in this case the microdentium shows many involuted forms. × 1,400.

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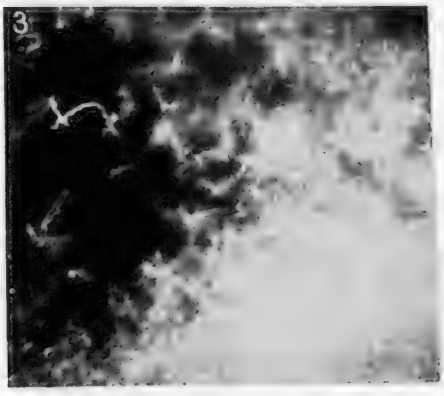
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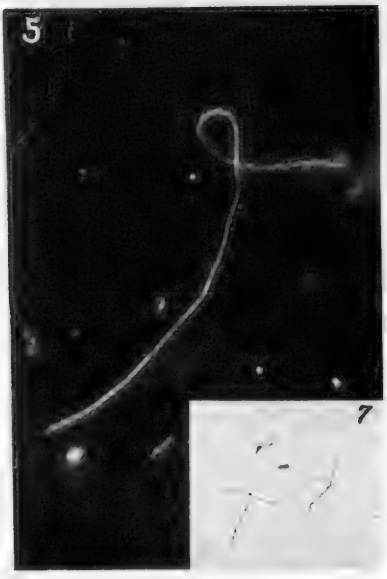
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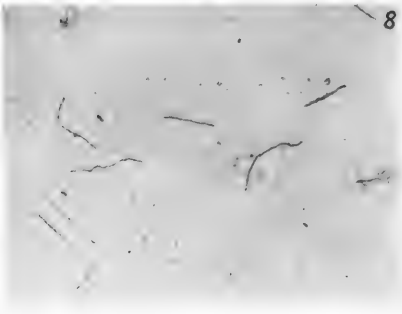
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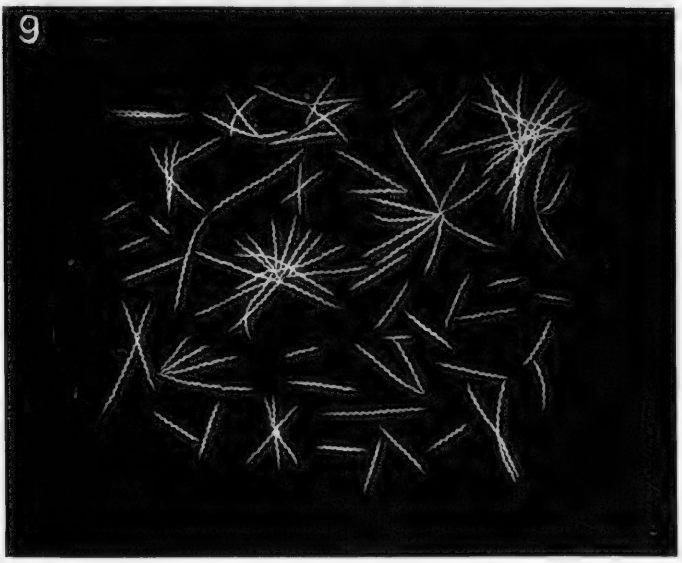
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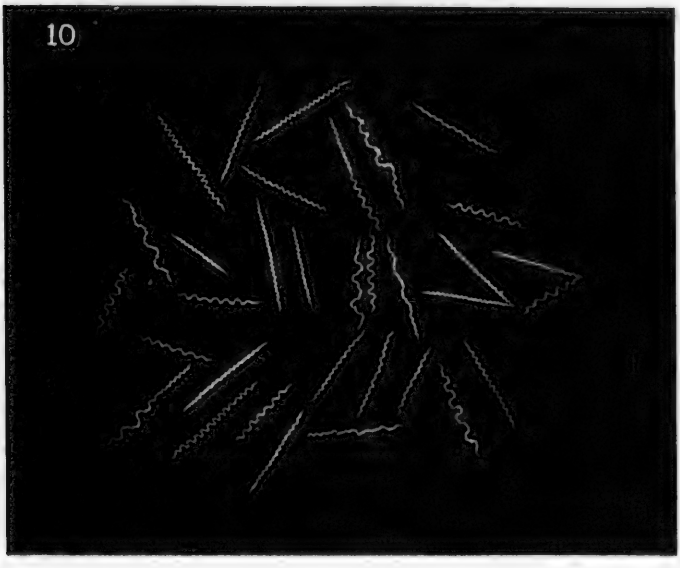
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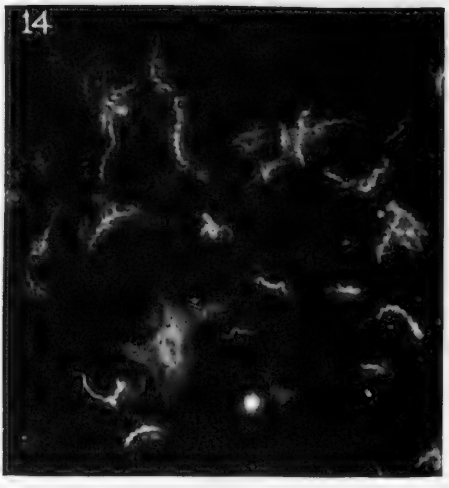
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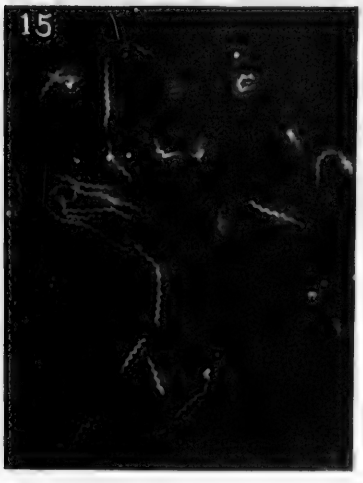
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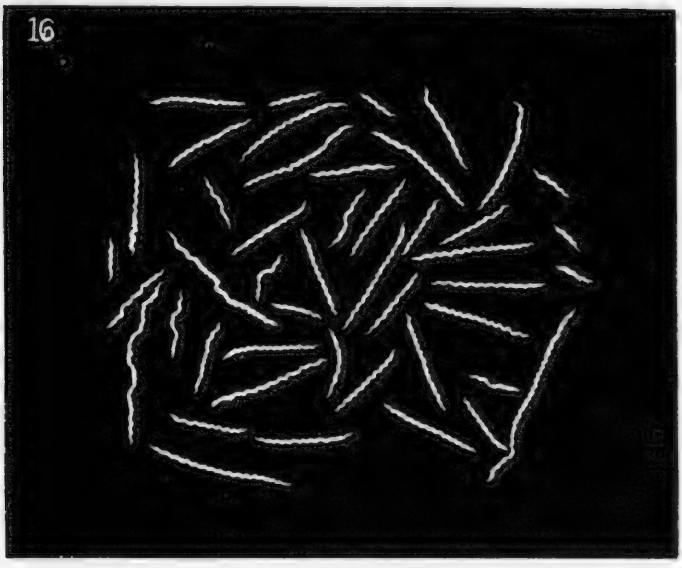
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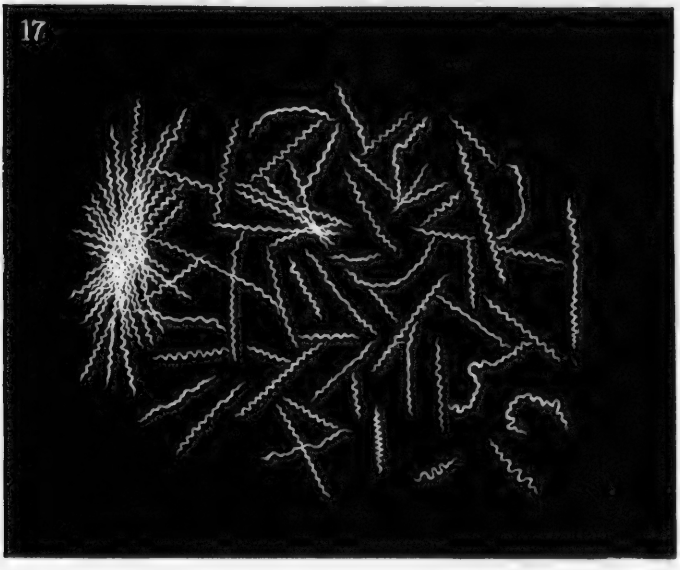
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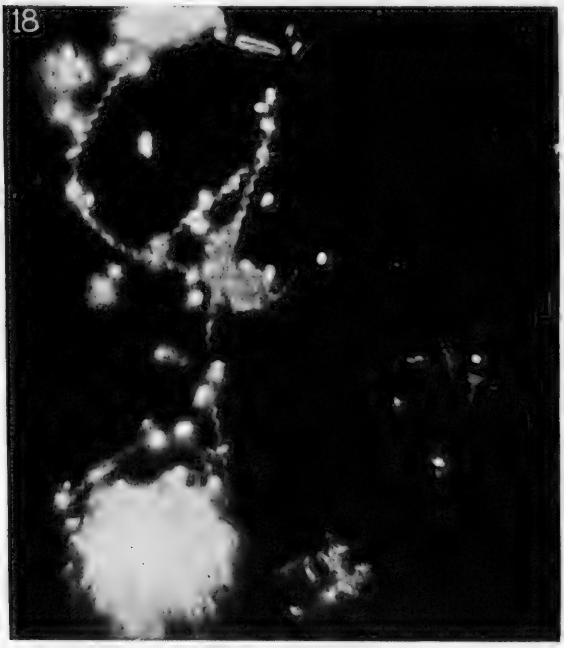
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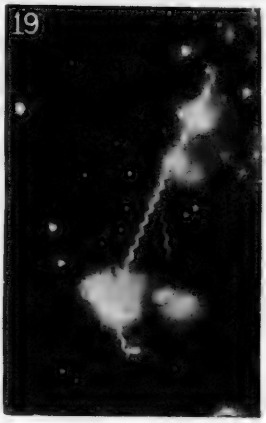
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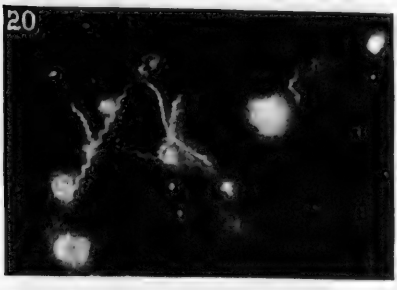
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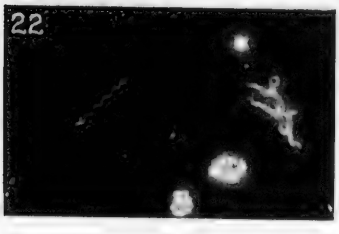
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PLATE 9.

FIGS. 11, 12, and 13. *Treponema macrodentium* from a young pure culture in a solid medium, seven days at 37° C. × 1,400.

FIGS. 14 and 15. The same from an older culture, many showing longitudinal division.

PLATE 10.

FIG. 16. A schematic reproduction of a dark-field view of a young solid culture (pure) of the macrodentium, ten days old at 37° C. × 1,400.

FIG. 17. *Treponema pallidum* from a pure solid medium, fourteen days old at 37° C. (For comparison.) × 1,400.

PLATE 11.

FIGS. 18, 19, 20, 21, 22, and 23. Involuted forms (?) of the macrodentium in an impure fluid culture. They show undeniable longitudinal division.

THE DIRECT CULTIVATION OF TREPONEMA PALLIDUM PATHOGENIC FOR THE MONKEY.*

By HIDEYO NOGUCHI, M.D.

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PLATES 12–14.

I have described, in a previous communication,[1] a method for the cultivation of *Treponema pallidum* from the testicular lesions of rabbits inoculated with human syphilitic material containing that organism. At that time I had failed to cultivate the pallidum directly from syphilitic lesions in man, a feat that has now been accomplished. In cultivating the organisms from the testicular lesions, I found it advantageous to employ first a fluid medium and later, after adaptation had taken place, to substitute a solid medium. This is a satisfactory method for the testicular lesions which are nearly free from contaminating bacteria, but it is not applicable to the highly contaminated primary lesions in human cases of syphilis.

Two conditions are all-important in the working out of the method of direct cultivation: first, the maintenance of strict anaerobiosis; and second, the property possessed by the pallida of migrating in the solid medium in which they are multiplying. To meet these conditions, I employed a high cylindrical layer of a solid medium consisting of two parts of 2 per cent. slightly alkaline agar and one part of ascitic or hydrocele fluid, at the bottom of which has been placed a fragment of sterile tissue, such as rabbit kidney or testicle. I have concluded that the tissue acts chiefly by removing the traces of oxygen that exist in the lower levels of the medium, and probably also by providing a special form of nutriment. The solid medium, when inoculated with a fluid containing pallida and bacteria, seems to keep the latter fixed to the inoculation stab while

* Received for publication, November 25, 1911.

[1] Noguchi, *Jour. Exper. Med.*, 1911, xiv, 99.

permitting the pallida to migrate to a distance where they may often be found in a pure or nearly pure state.

Comparisons have been made between the agar medium described and the gelatinized horse serum employed by Schereschewsky,[2] in favor of the former. The disadvantage of the gelatinized serum arises from the fact that the bacteria rapidly liquify it, and acids render it more permeable for bacteria. Hence it is very difficult to separate the spirochætæ from the contaminating bacteria.

DIRECT ISOLATION OF THE PALLIDUM.

Fifteen cubic centimeters of the medium described are placed into test-tubes measuring two by twenty centimeters. At the bottom is placed a fragment of sterile tissue. While rabbit kidney or testicle is preferable, human placenta, sheep testicle, or other sterile organ will suffice. The medium is prepared in the tubes, after the addition of the tissue, by mixing two parts of the melted agar at 50° C. and one part of ascitic or hydrocele fluid. After solidification, a layer of sterile paraffin oil three centimeters deep is added to prevent evaporation of the medium. A considerable number of tubes should be prepared at a time, and they should be incubated for a few days prior to inoculation, to determine sterility.

The material to be cultivated is obtained from lesions rich in the pallidum, such as chancre, condyloma, or skin papule, from which are snipped off, after cleaning with sterile salt solution, suitable fragments. These are immersed immediately into sterile salt solution containing in addition 1 per cent. sodium citrate. The fragment of tissue, which should be carefully preserved against drying, is then cut into small bits which are used as follows: one bit is emulsified in a mortar with the citrate solution; the others are inserted as such into tubes of culture medium, provided the emulsion shows, under the dark-field microscope, the presence of the pallidum.

Each of the half dozen bits of pallidum-containing tissue is inserted into a tube of the ascitic fluid tissue agar by being forced to the bottom by means of a thin blunt glass rod or heavy platinum loop, and into the same tube several drops of the emulsion are introduced by a capillary pipette also inserted deeply into the medium.

[2] Schereschewsky, *Deutsch. med. Wchnschr.*, 1909, xxxv, 835, 1260.

Care is taken to avoid tearing the medium, and about six tubes are inoculated from each specimen of tissue.

The tubes should be incubated at 37° C. constantly for two to three weeks before examination.

The inoculated tubes usually become diffusely opalescent and show, as well, a dense opaque growth of bacteria along the stab-canal and about the wall of the tube. When considerable gas is formed, the medium is torn. The cultures are now punctured in several places with a capillary pipette which is inserted deeply into the tube, and the withdrawn contents are examined under the dark-field. The rule is to find large numbers of the pallidum imbedded in the agar and hence immotile. Should the organisms be present in a space clear of agar, they show active motility. Bacteria are always mingled at this stage with the pallidum.

The impure culture is fetid and putrefactive. Purification is begun by inoculating about twelve tubes with the impure culture by means of a charged capillary pipette that is carefully inserted in the centre of the agar column quite to the bottom of the tube. The contents of the pipette are expressed by means of compressed air, for which purpose I employ a syringe. The medium must not be split. The inoculated tubes are again incubated at 37° C. for two or three weeks. The bacteria grow in a dense mass along the stab-canal and on the surface of the medium. The tubes are inspected, from time to time, and in some a faint hazy zone will be seen to radiate from the stab-canal towards the sides of the tube. This hazy appearance is caused, usually, by masses of the pallidum that are growing away from the bacteria. When this growth has spread sufficiently far away from the stab-canal another transfer is made.

The surface of medium is sterilized with sublimate alcohol that is poured away. The tube is marked about the middle with a diamond pencil, and to the cut a pointed red-hot glass rod is applied. The tube cracks and the upper half is removed. The exposed surface of agar is sterilized with sublimate alcohol, after which the remaining moisture is wiped away with sterile absorbent gauze. By gently bending the agar column, it cracks transversely and exposes a clear surface on which the hazy growth is readily seen. Now, without touching the central canal, a capillary pipette is introduced into the

haze and, after confirming, by dark-field examination, that it is caused by the presence of the pallidum, a series of inoculations are made in the manner described. It happens rarely that a pure culture is obtained on the first or second reinoculation; usually several reinoculations are required.

Not infrequently the growths of the pallidum are almost invisible. I am inclined to think that this is due to the quality of the culture medium. In this case, the dark-field microscope is the only reliable guide as to growth and situation in the culture of the pallidum. Again, the bacteria in the stab-canal may produce so much acid that it diffuses into the surrounding layer of medium where it causes a haziness capable of being mistaken for growth.

When ascitic or hydrocele fluid is not available, serum from the horse or sheep may be used for the purification process; they are, however, less satisfactory than the former fluids. It should be stated that I have found some specimens of ascitic fluid entirely unsuitable to the cultivation of the pallidum. When a favorable specimen has been obtained, it should be stored in sterile condition for future use.

PROPERTIES OF THE PURE CULTURES.

The pure cultures of the pallidum that I have studied have shown certain distinctive properties. First, the colonies of the pallidum that diffuse into the medium are never sharply, but always faintly visible, and the degree of visibility tends to be less in the pure than in the impure state. It seems to be unusual for the colonies to become discrete, although this has been observed when the sample of ascitic fluid has been very favorable. Second, the growth is most marked about the fragment of sterile tissue; I have not thus far succeeded in obtaining growth without fragments of tissue, although the medium used was similar in all other respects. In this important respect, the organisms that I have cultivated differ from those described by Mühlens[3] and by Hoffmann,[4] who obtained growth in a medium devoid of tissue. I have never observed my growths to cause any coagulation of the serum in a fluid medium, or to produce

[3] Mühlens, *Klin. Jahrb.*, 1910, xxiii, 339.

[4] Hoffmann, *Ztschr. f. Hyg. u. Infektionskrankh.*, 1911, lxviii, 27.

putrefactive odors, both of which are noted to occur by Mühlens and by Hoffmann.

When the medium is favorable, the first indications of growth appear in about seventy-two hours, and these increase gradually for two or three weeks, when the growth is at its height. The growth in a fluid medium takes place very slowly but continues for many weeks. The medium retains its fluid state indefinitely.

Morphology.—In young cultures, the greater part of the spirochætæ are short and actively motile. After growing for about two weeks, they attain the usual length of eight to twelve curves. Active motility is continued and the typical cork-screw movement is displayed. When the organisms are imbedded in the agar medium, they are immotilized. After several weeks, not only is the motility reduced, but the length has been increased (figures 1, 2, 3, and 5). The ends of the mature pallidum often show fine flagellum-like prolongations which are frequently perfectly curved, although sometimes they are devoid of curves (figure 6). The typical morphological characters are subject to disturbance by violent handling, as by the careless introduction into the growing mass of the capillary pipette or the presence of a trace of oxygen. Then the curves appear irregular, and straight forms occur. When irregular and atypical spirochætæ appear in certain tubes, they are due to the medium which is not strictly suitable, or to the presence of a trace of oxygen. To secure perfect and typical cultures, optimum conditions must be supplied.

Multiplication.—Schaudinn[5] described longitudinal division of the pallidum which he observed in two specimens. However, later investigators have described transverse division of the organism. It is important to ascertain which is the primary, or normal, and which the secondary, or abnormal, mode of division. That appearances suggesting both longitudinal splitting and transverse division are observable is admitted: the question relates to the significance of each process. Those who regard the longitudinal process as the normal one for multiplication look upon the transverse process as a last stage in the series of changes; while those who regard the transverse process as the normal one look upon the appearance of

[5] Schaudinn, *Arb. a. d. k. Gsndhtsamte.*, 1907, xxvi, 11.

longitudinal splitting as caused by the coiling of one spiral organism, still united by a thin end filament, about another part of the same organism which is undergoing transverse division.

The disputed points relate to unstained and stained preparations directly obtained from lesions in man or animals in which the possibility of artefacts can hardly be removed. In both cases, the conditions are not distinctly favorable. What is demanded are periods of development of the pallidum during the stage of active division, and this is provided in the pure cultivation at certain stages of growth. I have now studied about ten strains at various stages of proliferation and growth and under varying conditions of cultivation, so that I have formed an objective conception of the process.

The mode of division of the pallidum in pure cultures is such as to admit of ready following of the phenomenon under the dark-field microscope. During the period of active proliferation, numerous dividing individuals may be seen in a single field. Several classes may be constructed according to the stage of the process (figures 7, 8, 9, and 10): (*a*) the body is divided at about two thirds of its entire length; (*b*) the body is completely divided and one part is wrapped about the other with regular curves or flat waves; (*c*) one half the body is divided and the other not, and the divided half has separated widely so as to produce a figure Y; (*d*) the partially divided body diverges at angles to produce V forms of varying width. When an acute angle is formed, tenuous protoplasmic bridges may span the interval; (*e*) two bodies remain united by a thin filament, one of which may be of normal or abnormal form; (*f*) three or more individuals may be united in a series of fine filaments suggesting imperfect transverse segmentation; (*g*) finally, two blunt wavy individuals each about double the thickness of an ordinary pallidum may remain together (figures 11, 12, 13, 14, and 15). This last form differs from the others in possessing a few granules that are definitely arranged and aggregated into bands that run across the organism. Whether this condition is degenerative remains to be determined. The longitudinal form of division is met with in all the cultures irrespective of the length of the spirochætæ. During the dividing process, the curves may be shallow and merely wavy; but after the separation is accomplished, the curves

become deeper and regular. Motility is preserved during division, but is perhaps less active (figure 18, A, B, and C).

The various phases and varieties of the dividing process here described can best be followed when the spirochætæ are embedded at first in the agar medium that is pressed beneath the cover glass without disturbing their position. When the specimens are then liberated into a free space in which a fluid exists, the various phases can be readily traced as they pass from one to another, and in general in the order given, of the series described. Speaking broadly, the process is not rapid and requires about two hours for completion. The final result is two symmetrical halves, although asymmetrical division has been observed in rare instances.

PATHOGENIC PROPERTIES.

Treponema pallidum is a pathogenic organism. While its natural host is man, it is capable of implantation upon several higher animals, notably upon the monkey and the rabbit. I have already reported the successful inoculation of pure cultures of the pallidum, obtained from the testicular lesions of rabbits, to the testicles of normal rabbits.[6] Hoffmann[7] reported somewhat later the successful production of a specific orchitis by inoculating a pure culture derived immediately from human tissues. There is no real difference in these results, since originally all strains of the pallidum are human in origin. On the other hand, not all strains of the spirochætæ can be successfully implanted upon rabbits or other animals, when obtained directly from syphilitic lesions in man. Racial differences exist naturally, and probably can be produced artificially. The pallidum strains which I cultivated from the testicular lesions of rabbits and which were pathogenic for these animals, failed to produce skin lesions when inoculated into monkeys. But the strains described in this paper have been inoculated successfully into the skin of several monkeys.

In the experimental results to be reported, I employed three cultures of the pallidum derived from as many different human cases of syphilis and two species of lower monkeys, *Macacus rhesus* and

[6] Noguchi, *Jour. Am. Med. Assn.*, 1911, lvii, 102; *Jour. Exper. Med., loc. cit.*
[7] Hoffmann, *Deutsch. med. Wchnschr.*, 1911, xxxvii, 1546.

Cercopithecus callitrichus. The inoculations were made by applying ten day old pure cultures in ascitic fluid tissue agar to the freshly shaven, scarified surface of the skin over the eyebrows, and, also, to the scarified prepuce, scrotum, or labia. The cultures were smeared over the prepared surface, and several small injections were made with a very fine syringe. The final results of the inoculations cannot be stated yet, but the present conditions are given in the following protocols.

PROTOCOLS.

Macacus rhesus, A.—October 2, 1911. A ten day old pure culture of pallidum, strain No. 7 (from a chancre), was inoculated to both eyebrows and the left labium.

October 9. The traumatic reactions have all disappeared.

October 15. Several small nodules appeared on both eyebrows, and a pea-sized induration at the site of inoculation on the labium.

October 22. The small nodules still persist, and in some there is an increase in size; some are scaly and congested. No pallida are seen in the expressed juice. The labium is still indurated.

October 29. Two distinctly indurated nodules on the left, and one small one on the right eyebrow. On examination of the expressed juice under the dark-field, some active pallida are found. The nodule on the labium shows no pallida.

November 6. The indurated nodules on the eyebrows show superficial ulceration. The expressed juice contains numerous active pallida. The labium is still indurated, but no pallida are found.

November 11. The size of the nodules on both brows is increasing, showing a moderate degree of congestion. The labium shows only a slight induration. Photographed. The blood gives a positive Wassermann reaction (figures 16 and 17).

Macacus rhesus, B.—October 2, 1911. Inoculation made as above with the same culture.

October 9. The traumatic reactions have disappeared.

October 15. Several small indurations on the eyebrows. The skin of the scrotum shows marked induration at the site of inoculation.

October 22. The size of the nodules is increasing.

October 25. Death due to pulmonary tuberculosis. The indurated tissues were excised and examined for the pallida, but none were found.

Macacus rhesus, C.—October 2, 1911. Inoculation made as in monkey B.

October 9. The traumatic reactions have disappeared.

October 15. One nodule on the left, and three small nodules on the right eyebrow. The labium is moderately indurated.

October 29. The nodules on the eyebrows have become larger and congested. The labium is indurated.

October 31. Death from pulmonary tuberculosis. Examination of the indurated tissues from the eyebrows shows numerous pallida.

Macacus rhesus, D.—October 2, 1911. A ten day old pure culture of pallidum, strain No. 8 (from a condyloma), was used for the inoculation.

October 9. The traumatic reactions have all disappeared.

October 15. Several small indurations on both eyebrows. The labium shows marked induration at the site of inoculation.

October 22. No further changes.

October 29. The nodules are somewhat larger and fewer in number. The induration of the labium is still about the same. The expressed juice of the nodules on the brows shows a small number of active pallida.

November 11. The nodules are becoming smaller in size, and the labium is healing up. No pallida.

Macacus rhesus, E.—October 2, 1911. Inoculation as above.

October 9. All traumatic reactions have disappeared.

October 15. No change.

October 22. No induration.

October 29. No change.

November 11. Still no change.

Macacus rhesus, F.—October 2, 1911. Inoculation with a ten day old pure culture of pallidum, strain No. 9 (from a rectal condyloma).

October 9. The eyebrows are smooth and show no induration.

October 15. Several small indurations on the eyebrows.

October 22. The nodules are disappearing.

October 29. The nodules have disappeared.

November 10. No further change.

November 11. Death from pulmonary tuberculosis.

Cercopithecus callitrichus, G.—October 2, 1911. Inoculations with the same culture as in monkey F.

October 9. The eyebrows are clean.

October 15. There are several small nodules on both brows.

October 22. The nodules are increasing in size.

October 29. A distinctly raised nodule is seen near the corner of the left eyebrow. The small nodules are disappearing.

November 6. The nodule on the left brow has become very much congested and covered with a scaly crust. Under the crust, the nodule is barely covered with a new epidermis, which by slight scraping caused exudation of a clear serous fluid. Examination of the exudate showed a few active pallida.

November 11. The nodule is still much indurated and congested, but the surface is well covered. By gentle scraping, a serous exudate containing pallida escapes from the nodule. The blood gives a positive Wassermann reaction. Photographed.

From these results it can be inferred that pure cultures of *Treponema pallidum* are capable of causing, in certain lower species of the monkey, lesions of a characteristic form and history that resemble the lesions caused by inoculations of the spirochætæ-containing serum from a primary syphilitic lesion. Coincidentally, the development of the Wassermann reaction in the successfully inoculated monkeys has been observed.

CONCLUSIONS.

A method for the direct cultivation of *Treponema pallidum* from human syphilitic lesions, by the employment of a solid medium, has been described. By means of it, three of the four strains worked with were successfully cultivated.

The several pure cultures agree in morphological and cultural characters, grow only in the presence of sterile tissue under anaerobic conditions, and do not produce putrefactive odors. The morphology is typical under optimum cultural conditions; it becomes atypical when the conditions are unfavorable.

In cultures, *Treponema pallidum* multiplies by longitudinal division. The process is usually symmetrical but occasionally appears to be asymmetrical.

Inoculation of the pure cultures into the skin of two species of lower monkeys was followed by the production of lesions resembling the primary syphilitic lesion occurring in man and those caused in the monkey by inoculation of spirochætæ-containing serum from human sources.

During the course of the positive inoculation in the monkey, the blood develops the property of giving a positive Wassermann reaction. Thus the relation of *Treponema pallidum* to this reaction is supported, and the identity of the cultivated strains with the species found in human syphilitic lesions established.

EXPLANATION OF PLATES.

PLATE 12.

FIG. 1. *Treponemata pallida* from a young pure culture in an ascitic agar tissue medium, four days old at 37° C. Dark-field. × 1,400.

FIGS. 2 and 3. The same after two weeks.

FIG. 4. *Treponemata pallida* from a chancre. (For comparison.) × 1,400.

FIG. 5. *Treponemata pallida* from a pure culture in an ascitic agar tissue medium, two weeks at 37° C. The pallida in free space show typical morphology.

FIG. 6. A short pallidum with flagella-like projections at both ends. × 1,400.

FIGS. 7, 8, 9, and 10. An ascitic agar tissue culture (pure) showing various phases of longitudinal division. × 1,400.

PLATE 13.

FIG. 11. A mass of dividing pallida from a pure culture in solid media. × 1,400.

FIGS. 12 and 13. Peculiar forms of dividing pallida from a pure culture in solid media. × 1,400.

FIGS. 14 and 15. Dividing forms of pallida in fluid cultures.

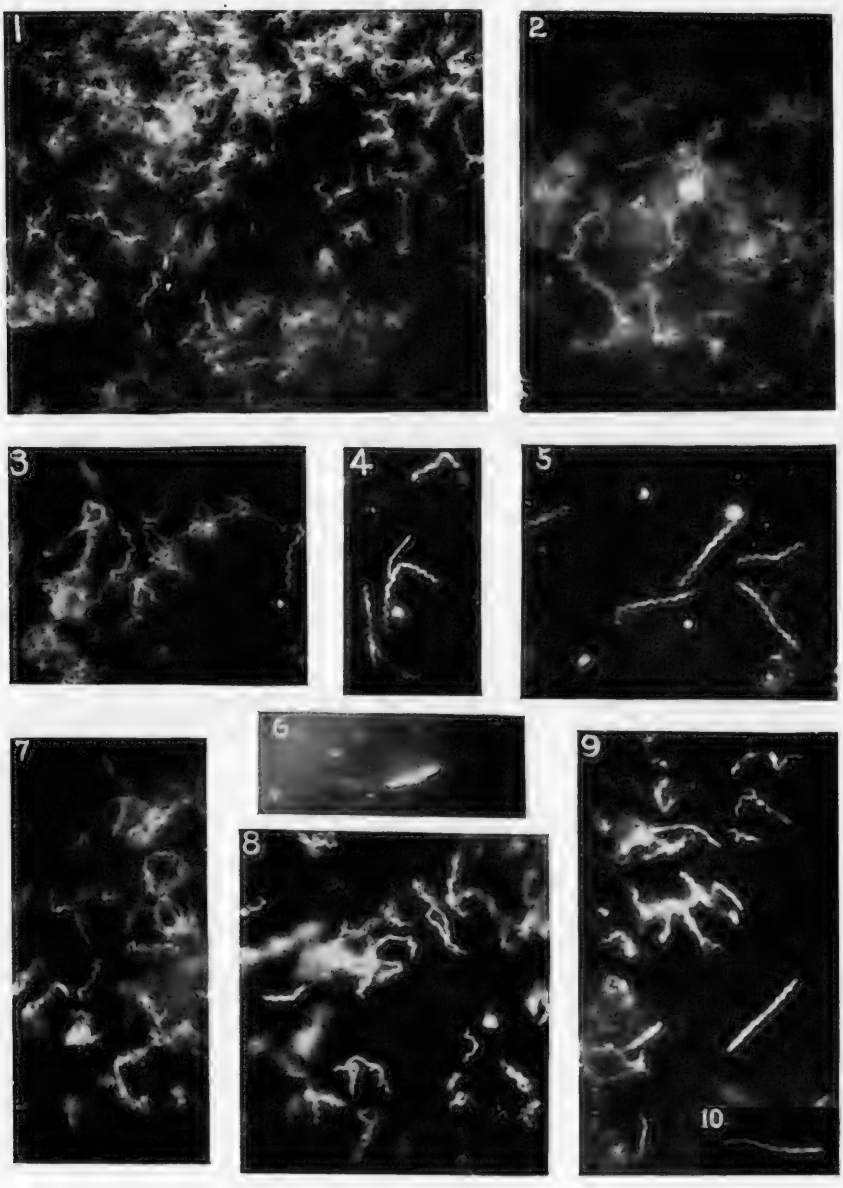
FIG. 16. *Macacus rhesus, A,* with primary indurations on the skin above the eyebrows produced with a pure culture of the pallida. Photographed after forty days.

FIG. 17. A pallidum in the expressed juice of the lesions of the same monkey.

PLATE 14.

FIG. 18. A schematic reproduction of different phases of longitudinal division of *Treponemata pallida* in solid culture (pure). The drawings were made from observations on living organisms under the dark-field microscope. The description of these forms will be found in the text.

A = symmetrical division; *B* = asymmetrical division; *C* = irregular forms.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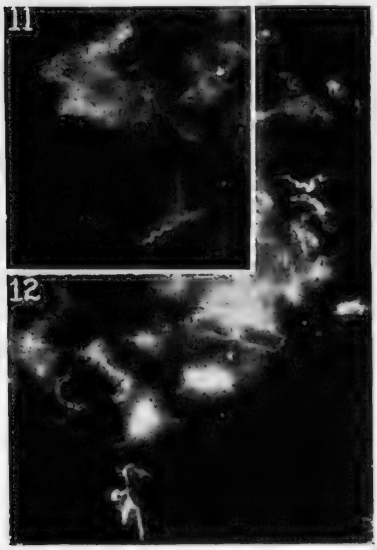
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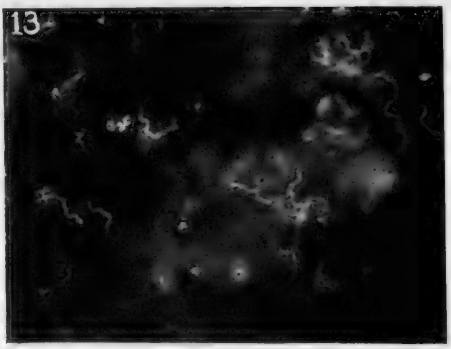
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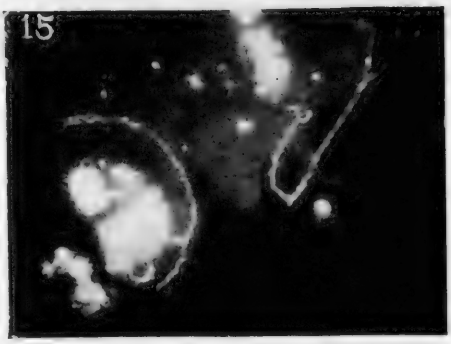
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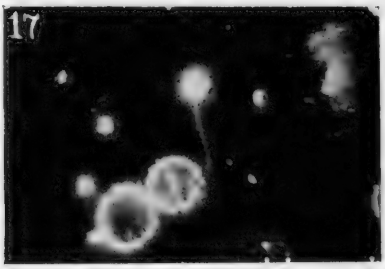
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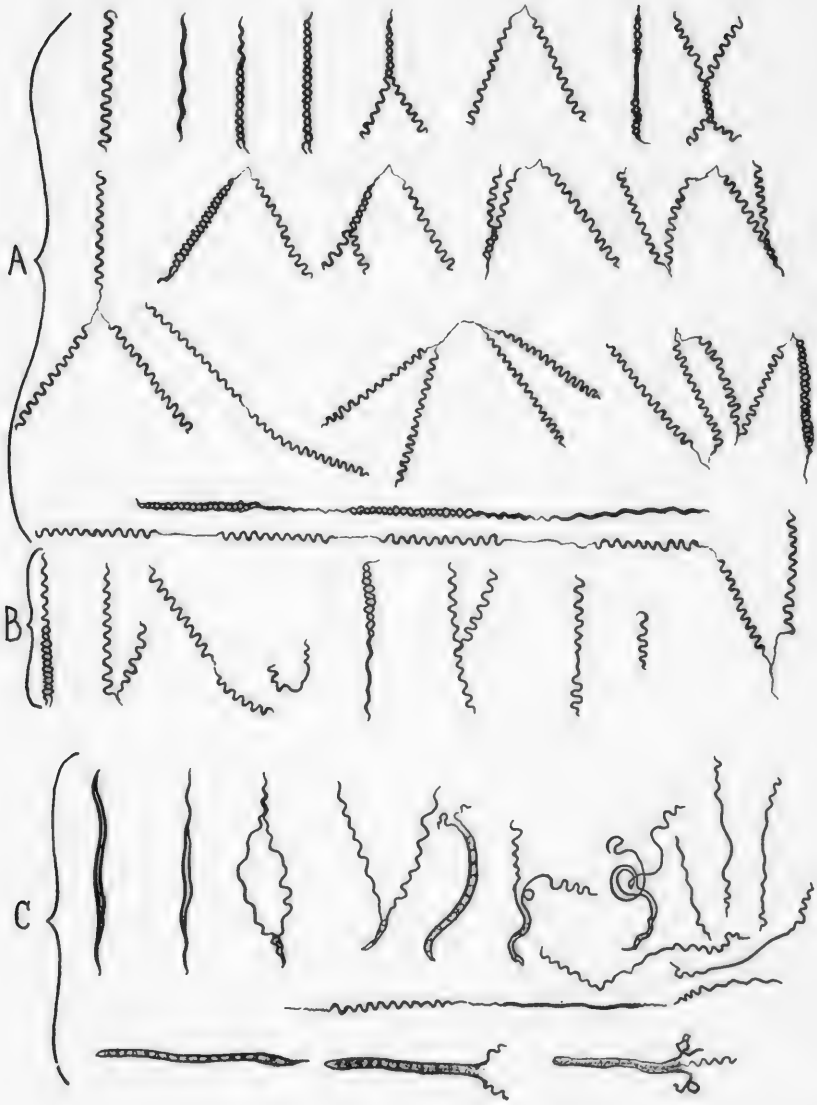
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THE ISLANDS OF LANGERHANS AFTER LIGATION OF THE PANCREATIC DUCTS.*

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PLATES 15 AND 16.

Many recent papers on the islands of Langerhans in the pancreas refer to the effects produced by ligation of the pancreatic ducts. Mankowski, Pratt and Spooner, and others state that after ligation the islands of Langerhans show no especial immunity, acinar and islet cells alike undergoing necrosis. On the other hand, such investigators as Vassale, Schulze, Ssobolew, and De Witt, affirm the persistence of the unaltered islet cells when the acinar cells have become atrophied. Lombroso states that after occlusion of the ducts some acinar as well as islet cells persist, while Dale holds that the preëxisting islets show no especial resistance, but that some of the acinar cells which escape atrophy assume the islet form. Laguesse shows the persistence of islet tissue but adds that after occlusion of the ducts new islets can bud at the expense of the excretory canals. The question of the persistence of the islets is of special importance when regarded from the standpoint of the relation of the islands of Langerhans to glycosuria. For, while the observations of Mering, Minkowski, and others showed that glycosuria invariably resulted from complete removal of the pancreas, it has since generally been shown that ligation of the ducts is not followed by glycosuria.

In a paper on the relation of the islands of Langerhans to glycosuria, MacCallum[1] describes an experiment which was made in order to test this point. A distal portion of the pancreas of a dog, comprising about one third of the whole gland, was separated, and the ducts were ligated. Seven months later the isolated portion was

* Received for publication, November 1, 1911.

[1] *Bull. Johns Hopkins Hosp.*, 1909, xx, 265.

found to be reduced to a mere thickening of the mesentery, in which, however, abundant blood vessels could be seen. At that time, the normal remainder of the gland was completely removed. Glycosuria followed, but was very slight and transient, lasting only three days, after which time a very considerable tolerance for sugar was attained. Twenty-one days later, the abdomen was again opened and the opalescent area in the mesentery removed. This was followed by extreme glycosuria. Unfortunately, the thyroid was removed some days later, after which the glycosuria disappeared.

Microscopically, the portion of the mesentery which had been removed showed remnants of the atrophied pancreas consisting of shrunken ducts and masses of cells. The cells were morphologically like the islet cells. Nevertheless, their appearance was not so typical as to eliminate all doubt in this respect.

The present study was undertaken for the purpose of answering, if possible, the question whether the groups of cells described by MacCallum and others were really persisting islands of Langerhans. It was thought that this could be shown by means of some differential stain which would bring out a sharp contrast between the secreting cells of the pancreas and the cells of the islands of Langerhans; a contrast so sharp that there could be no possibility of mistaking their identity, even when seen under completely altered conditions.

These requirements seemed to be fulfilled by Lane's[2] method in which Bensley's neutral gentian is used after an alcoholic or aqueous chrome sublimate solution. The results after fixation in the alcoholic solution were generally unsatisfactory; the best were obtained from a slight modification of the aqueous chrome sublimate method. The results, however, did not entirely agree with those described by Lane. Practically all the islet cells showed granules after the aqueous fixation, and the larger cells, instead of failing to show them, were often those which appeared most crowded with granules. It seems possible that, as in the acinar cells, the difference in the number and arrangement of the granules is simply an indication of the functional activity of the cells.

Figure 1 shows the pancreas of a normal guinea pig which had

[2] *Am. Jour. Anat.*, 1907–8, vii, 409.

been removed from the anesthetized animal during life and after fixation in the aqueous solution, stained with neutral gentian. The islet cells are filled exclusively with very fine dark blue granules, while the dark zymogen granules of the acinar cells stain a reddish brown. The acinar cells never showed blue granules. It could be expected, therefore, that the islet cells would be recognizable as such if this distinctive character was maintained after ligation of the ducts.

Guinea pig D was operated upon in the same way as described for the dog in MacCallum's paper, the ducts being tied off by means of two differently colored threads and the area between divided so that the distal portion of the pancreas was quite separated from the rest. Three weeks later, when both portions of the pancreas were removed, that distal to the ligature was found considerably shrunken and surrounded by adhesions.

Microscopically, the isolated portion showed one or two areas of necrosis evidently due to disturbance of the blood supply. Throughout the remaining areas there was a greatly increased amount of connective tissue in which were embedded the shrunken, though still recognizable, pancreatic lobules. While much shrunken, many of the cells still showed the typical zymogen granules. The islands of Langerhans, their cells loaded with dark blue granules, stood out in striking contrast, as shown in figure 2. They appeared normal and were generally quite sharply marked off from the degenerating acinar cells. Their blood supply was evidently abundant.

Guinea pig A, a young animal of moderate size, was operated upon by Dr. MacCallum, on December 30, 1909, at which time the distal portion of the pancreas, about one third of the gland, was separated from the common mass and the ducts were ligated. The animal showed no ill effects, and indeed, grew to a large size and remained in perfect health for nearly fifteen months, until March 23, 1911, when it was killed. The isolated portion of the pancreas was found to have been converted into a cyst-like duct surrounded by masses of fatty tissue in which were embedded opaque dots, or strands, the only traces remaining of the former pancreatic tissue. The remainder of the pancreas appeared normal.

Microscopically, there was to be found no trace of acinar cells.

Figure 3, which is a drawing under low power of a section of the fatty mass, shows clearly that the remains of the pancreatic tissue consist exclusively of small masses of cells which stain blue. Such a mass is shown under higher magnification in Figure 4, in which it can be seen that the blood supply is ample. Close examination, by the aid of Bensley's stain, shows that the cells are filled with fine blue granules characteristic of the islet cells, and leaves no doubt that they belong to persisting and actively functioning islands of Langerhans. In some of the islets, as is shown in figure 4, groups of cells or single cells are scattered indifferently about, in which the granules are very closely packed, so that the cytoplasm of the cell assumes a darker color. These cells, on the whole, appear slightly larger than those with fewer granules. Their nuclei usually contain from one to three fairly large chromatic masses, while the nuclei of the smaller cells most frequently contain more numerous and smaller granules. It may be repeated that no trace whatever of acinar cells containing zymogen granules and showing the yellowish cytoplasm characteristic of these cells with this stain is to be found in the tissue of this atrophied portion of the pancreas.

Several conclusions may, perhaps, be drawn from this preliminary study. First, that the contrast in the staining reaction between the granules of the acinar and the islet cells, as shown by Lane, can be confirmed. Secondly, that during the atrophy of the acinar cells and even long after the last traces of them have disappeared, the islet cells remain morphologically unaffected and filled with the granules which are peculiar to them. Taken together with the experiments of MacCallum, this seems to offer strong evidence in support of the view that the islands of Langerhans are especially, if not exclusively, concerned with the maintenance of the normal carbohydrate metabolism.

EXPLANATION OF PLATES.

PLATE 15.

FIG. 1. Pancreas from a normal guinea pig. The zymogen granules of the acinar cells stain reddish brown; the granules of the islet cells stain deep blue.

FIG. 2. An island of Langerhans from the isolated portion of the pancreas, twenty-one days after ligation of the duct. The acinar cells are undergoing atrophy but still show a few zymogen granules. The islet cells are filled with blue granules.

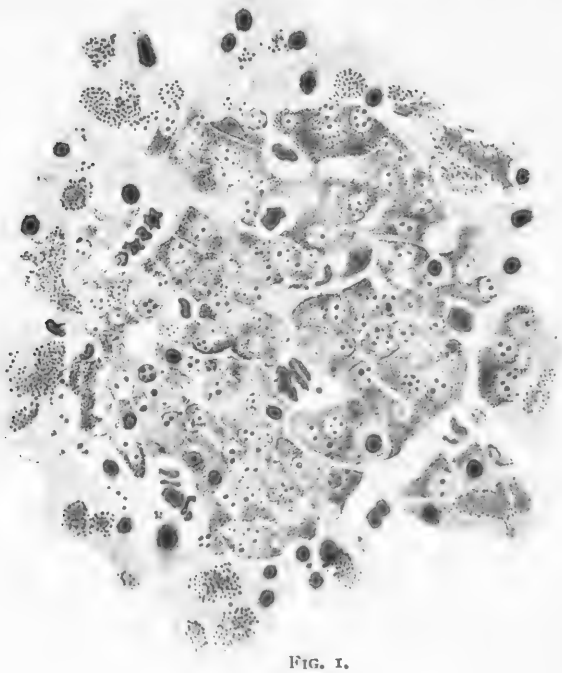

FIG. 1.

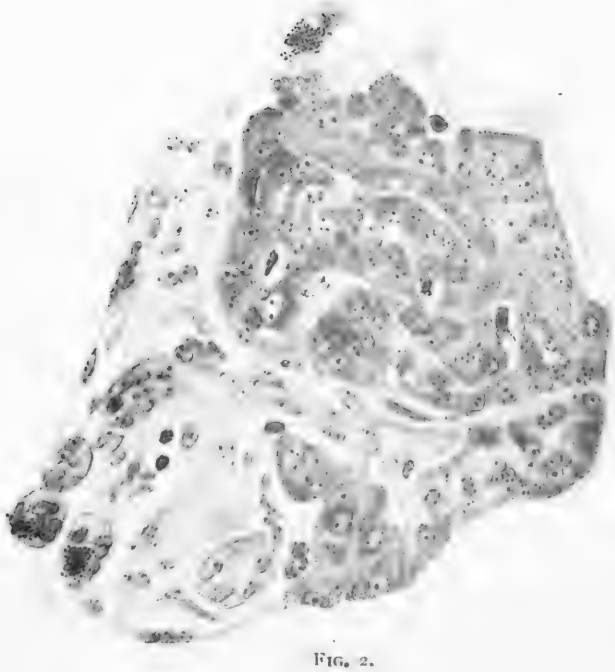

FIG. 2.

FIG. 3.

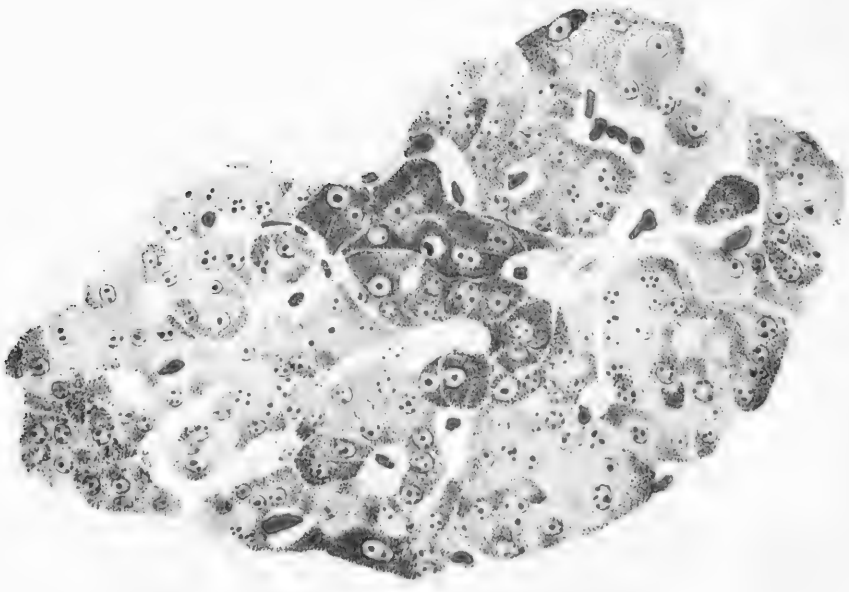

FIG. 4.

PLATE 16.

FIG. 3. A section of atrophic pancreas from guinea pig A, fifteen months after ligation of the duct. The pancreatic tissue is reduced to small islands which stain blue with neutral gentian.

FIG. 4. One of the islands from the specimen referred to in figure 3, enlarged. The drawing shows the persistence of islet tissue alone, some of the cells being more closely packed with granules than others.

The same technique was used in all cases; namely, Zenker's fluid without acetic acid, and neutral gentian violet.